THE UNIVERSITY OF ALBERTA

POPULATION STUDIES ON MOSQUITOES

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Population Studies on Mosquitoes" submitted by Yoshito Wada in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The seasonal fluctuations of each instar larvae and pupae of *Culiseta inornata* (Williston) in a particular pool near the University of Alberta were investigated and an attempt to estimate the mortality of the aquatic stages was made. The data for the collections of adults and larvae of 26 species of mosquitoes found around Edmonton indicate that the black-legged mosquitoes of subgenus *Ochlerotatus*, genus *Aedes* are earlier-appearing species than others. The distribution pattern of mosquito larvae was firstly demonstrated to follow a negative binomial distribution with a common value of constant k for various density levels. Based on this distribution pattern, a sequential sampling technique was applied to classify a mosquito population into one of three predefined density levels. This was considered useful in deciding whether or not control is necessary, and in evaluating whether or not control has been successful over a wide area in a relatively short time.

The effect of larval density of *Aedes aegypti* (L.) on larval development and the size of resulting adults was studied in the laboratory, and it was shown that high larval density is associated with high larval mortality, long larval period, and small size of resulting adults. When the larval density is very low, relative wing length seems to be smaller. The effect of the density is considered to be expressed through increased stimulation of larvae by mutual contacts.

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PART I. POPULATION STUDIES ON EDMONTON MOSQUITOES

1. GENERAL INTRODUCTION

The City of Edmonton has been engaged in the control of mosquitoes and has reduced the mosquito population greatly in the city (see Klassen and Hocking, 1963 and 1964). However, there are still some problems to be solved. They include precisely when and how the insecticidal applications should be made for the effective and economical control of mosquitoes, how far the larvicide should be applied beyond the city limits, and so on. For the settlement of them, extensive fundamental studies are required. This report deals with the studies conducted in 1964 to approach the problems from an ecological point of view.

In Section 2, some data on the bionomics of Edmonton mosquitoes are given. Section 3 gives the distribution pattern of mosquito larvae within a particular pool, which will provide the basis for the sampling of mosquitoes. The possibility of the application of sequential sampling technique to mosquito larval populations is discussed in Section 4, based on the above distribution pattern. In Section 5, suggested studies on some ecological subjects are discussed. Work along these lines should provide better control of Edmonton mosquitoes.

2. BIONOMICS OF EDMONTON MOSQUITOES

2.1. Mosquito surveys and identification

Three types of mosquito surveys were made in 1964. Firstly larval (and pupal) surveys were made at pools in various environments around Edmonton, mostly westward, from April to July. The number of

dips at each pool was not recorded, except for a few pools for determining the distribution pattern of larvae per dip, which will be mentioned in Section 3. However, care was taken in catching mosquitoes so as to represent the mosquito fauna there; only a few dips were made at pools with high mosquito density and many dips, sometimes more than 50, at pools with low density. The results are given in Section 2.4.

Secondly collections were made of adult mosquitoes, which came to feed on me, around a particular pool near the University of Alberta at approximately one week intervals. The results are also given in Section 2.4.

Thirdly larval surveys were made at the pool mentioned above. The pool harboured almost exclusively Culiseta inornata (Williston) and the seasonal changes of immature stages are given in Section 2.3.

The larvae collected were reared in the laboratory to the fourth instar or to adults, and identified. Some specimens were separately reared to obtain the adults with associated larval skins to facilitate determining the species.

The identification of larvae followed Carpenter and LaCasse (1955) and Rempel (1950). Adults were identified mostly after Carpenter and LaCasse (1955) and Rempel (1953). However, it was often difficult to separate them to species, especially rubbed specimens of black-legged female Aedes. In such cases, and even for good specimens, the post-coxal scale patch (between the anterior coxa and the sternopleuron), mesepimeral scale patch, scales of probasisternum, and tarsal claws

were useful characters (Beckel, 1954; Vockeroth, 1954).

2.2. Notes on some species

Aedes communis (DeGeer) and *Aedes intrudens* Dyar

A. communis and *A. intrudens* are black-legged species lacking the post-coxal scale patch. The adult female of *A. communis* is usually separable by the contrasting stripes on the scutum from *A. intrudens* with a uniformly colored scutum. However, in some specimens of *A. intrudens* the scutum shows indications of paired median brown stripes, and those specimens, particularly when the scales on the scutum are not complete, are sometimes hard to distinguish from *A. communis*.

After examinations of 38 females of *A. intrudens* and 32 of *A. communis*, some of which were associated with their larval skins, it was found that, as described by Carpenter and LaCasse (1955), mesepimeral scales reach near lower margin in *A. communis*, but in *A. intrudens* the lower third or fourth is bare. This seems to be a most useful character to separate them. Other characters, which might be used, are the number of lower mesepimeral bristles and the color of the base of the costa. The lower mesepimeral bristles vary in number in both species, but in the present specimens *A. intrudens* has a smaller number of bristles, ranging from 0 to 3, than *A. communis*, which has 2 to 7 bristles. White scales at base of the costa are absent, or if present very few in number, in *A. intrudens*; they are present in *A. communis*.

Aedes hexodontus Dyar and *Aedes punctor* (Kirby)

The adults of these two species are very similar to each other, however the larvae are distinct. According to Beckel (1954), the probasisternum has white scales and an extensive patch of white scales is seen at the base of the costa in *A. hexodontus* taken in the field at Churchill, Manitoba; on the other hand in *A. punctor* taken there scales on the probasisternum are reduced to a few and there are no white scales at the base of the costa or rarely one or two. These characters were found useful to separate specimens of these species taken near Edmonton also, by examination of females associated with their larval skins.

Knight (1951) recognized two varieties in each species: "type *hexodontus*" and "tundra" variety in *A. hexodontus* and "type *punctor*" and "tundra" variety in *A. punctor*. The scutum of females has a broad median dark stripe which may be narrowly divided in "type *hexodontus*" and "type *punctor*", on the other hand in "tundra" variety of both species the median dark band is absent or not well defined.

Of the females of Edmonton *hexodontus* collected or reared from larvae, 9 are "tundra" variety and one is "type *hexodontus*" variety. The latter was collected as an adult on June 7, 1964. In addition to these, I have another female specimen of "type *hexodontus*" variety, which was reared from a larva taken near Jasper, Alberta, on May 16, 1964. The associated larval skin shows that head hairs 5 and 6 are both double, which agrees with the description given by Knight (1951) for "type *hexodontus*" variety.

As for *A. punctor*, the many larvae and 18 females, which were collected as adults or reared from larvae, are considered all "type *punctor*" variety.

Aedes niphadopsis Dyar and Knab

A larva of this species was taken from a collection of small scattered pools in a pasture near a creek, about 20 miles west of Edmonton, on June 7, 1964, and reared to a female adult. This record is new to Canada (Pucat, 1964).

Aedes pullatus (Coquillett)

This is a species that lacks the post-coxal scale patch, and bears a distinct hypostigial scale patch. The distribution in Alberta seems to be limited mostly to mountainous regions. I collected many larvae from snow-melting pools in Jasper National Park on June 21, 1964, but no specimens were encountered around Edmonton.

2.3. Seasonal fluctuation and mortality of immature stages of Culiseta inornata (Williston)

Observations were made on the changes in abundance of each instar larvae and pupae of *Culiseta inornata* (Williston) throughout a season at approximately one week interval in 1964 at a pool, ca. 10 x 3 m, near the University of Alberta. The pool is situated on the south bank of the North Saskatchewan river, and receives little sunlight because of tall vegetation such as poplars around it. For this reason, ice remained at the bottom of the pool as late as May 8, and the water temperature was relatively low throughout the summer; the maximum water temperature

was only 18.3 C on August 17.

On each day, larvae and pupae were sampled with a dipper usually ten times, but when necessary, 20 or 50 times, and the numbers of each instar larvae and pupae were recorded. The population of mosquitoes in the pool consisted of only *C. inornata*, as far as the fourth instar larvae were examined. However, from some egg rafts collected at the pool on July 6, there emerged some adults of *Culiseta alaskaensis* (Ludlow) in addition to *C. inornata*; this indicated that a small number of egg rafts, probably one, of the former species was mixed in the collection of the egg rafts. Therefore, some *C. alaskaensis* may have bred also in the pool, but, even so, the number seems to have been negligibly small.

Egg rafts were first encountered on May 25, and oviposition continued until August 10. The number of egg rafts per dip and the observation for the rafts on the water surface of the pool show that the peak of oviposition activity of *C. inornata* was in the first half of June.

The seasonal distribution for each instar larvae and pupae is shown in Fig. 1. The first individuals of larvae in the first, the second, the third, and the fourth instar, and pupae were encountered on May 25, June 2, June 16, and June 22, respectively. The peak in numbers of first instar larvae was June 8, and with the progress of the development the time of each peak became successively later; the peak for pupae was on July 6. The period between the peaks of first instar larvae and of pupae is about one month. This seems to be the time required for

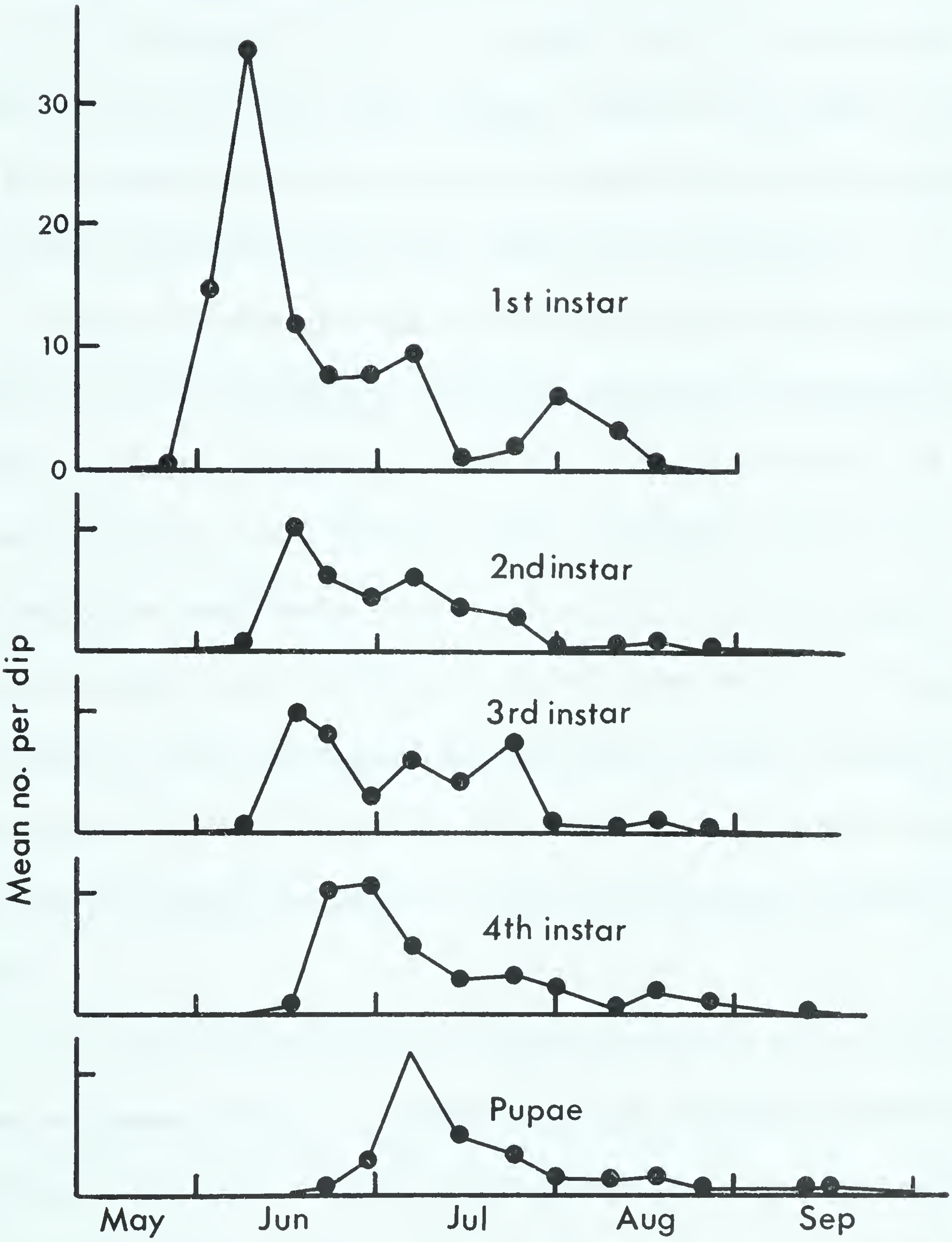

Fig. 1. Mean number of each instar larvae and pupae of *Culiseta inornata* per dip.

C. inornata to develop from the first instar larva to the pupa; the mean temperature was 11 C during the period.

The emergence of adults is thought to have occurred most actively shortly after the peak of pupae, that is in the middle of July. This time of peak emergence was ascertained by the fact that many pupal skins were observed on the water surface on July 14 and 23.

It has been reported that the duration of the larval stage of mosquitoes such as *Anopheles quadrimaculatus* Say and *Aedes aegypti* (L.) is affected by temperature, nature and amount of food, and density of a population (e.g. see Horsfall, 1955). Therefore, the above period of one month at mean water temperature of 11 C will be changed to some extent according to the conditions in a pool, even when the temperature is the same. Also, the remarkable difference in water temperatures within a pool (Haufe, 1957) may influence the data. However, the difference does not seem to be great, as most larvae inhabit similar environments.

The area surrounded by the abscissa and the curve for each instar larvae and pupae in Fig. 1 is dependent on the relative abundance and also on the duration of each instar. In the laboratory at ca. 23 C, an egg raft of *C. inornata* was reared to adults, and mean periods for each instar larvae and pupae were obtained. If it is supposed that these mean periods are kept unchanged also in the present field data, we can get the relative abundance by dividing the calculated area from Fig. 1 by the mean period. The results are given in Table 1. It is recognized from the table that the

reduction in the relative abundance is remarkable between the first and the second instar larvae, and between the third and the fourth instar larvae.

Table 1. Relative abundance of each instar larvae and pupae of C. inornata in the field.

		Larvae				
		1st	2nd	3rd	4th	Pupae
Area (days x no. of individuals) in Fig. 1	(A)	718	275	311	292	220
Mean period (days) in the laboratory	(B)	2.9	2.0	2.3	4.2	3.5
Relative abundance in the field	(A/B)	248	138	135	70	63

The survival rate from the first instar larvae to the pupae is estimated at 63/248 x 100 = 25%. Thus we get a mortality of 75% for the aquatic stages of C. inornata, or slightly higher, as the mortality in the earlier half of the first instar larvae and the latter half of the pupae is not included in the above calculation.

The reliability of the above calculation depends on how effectively the material was sampled from the pool and how close the relative mean duration for each instar larvae and pupae obtained in the laboratory is to that in the field. As will be mentioned later, the number of larvae plus pupae of mosquitoes per dip follows a negative binomial distribution having a larger variance than a random distribution. This means that a larger number of dips is required to estimate the population effectively,

and the number of dips may be too small in the present field data. As mentioned earlier, the mean duration of larval stage is affected by temperature, food, and population density, but perhaps little affected in pupae by the last two factors. Therefore, it is rather difficult to compare the values in the laboratory with those in the field. Another difficulty is that the temperature in the field changes daily and seasonally. Nevertheless, the above method of estimating the mortality is of value as a first approach to this important subject.

In any case, it seems that the mortality in the aquatic stages of *C. inornata* is fairly high in the field. The factors responsible for this are not known. However, physiological disorder or a sort of disease is supposed, as some dead larvae were found and all attempts to find predators in the pool failed.

2.4. Seasonal occurrence of Edmonton mosquitoes

Table 2 gives the number of larvae (and pupae) collected around Edmonton and the number of collections in which each species was found. Mosquitoes were encountered at 30 pools out of more than 60 examined. Since the number of dips varies from pool to pool, the number of larvae shown in the table does not represent exactly the relative abundance of each species. However, the main features of seasonal appearance are clearly seen.

The results of the collections of female mosquitoes, which came to feed on me, around a pool on the south bank of the North Saskatchewan river near the University of Alberta are given in Table 3. This table

Table 2. The total number of larvae and pupae collected around Edmonton, and the number of collections (within parentheses) in which each species was found.

Species	April early	April late	May early	May late	June early	June late	July early	July late	Total
Anopheles									
earlei				58(2)		4(2)	8(1)		70(5)
Culex									
tarsalis								1(1)	1(1)
territans						1(1)	5(1)	3(2)	9(4)
Culiseta									
alaskaensis								1(1)	1(1)
inornata					20(1)	436(2)	7(2)	158(2)	621(7)
morsitans					2(1)				2(1)
Aedes									
campestris		37(1)							37(1)
canadensis		1(1)							1(1)
cataphylla	19(3)		3(1)						22(4)
cinereus		1(1)	3(2)	4(1)	2(1)				10(5)
communis	10(3)	34(1)	16(2)						60(6)
dorsalis				1(1)	1(1)	2(1)			4(3)
excrucians	5(2)	6(1)	6(1)	13(1)	2(1)				32(6)
fitchii	31(2)	65(2)	3(1)	47(1)	2(1)				148(7)
flavescens				3(1)	1(1)				4(2)
hexodontus	3(1)	3(2)	1(1)						7(4)
implicatus	23(3)	24(3)	20(1)	1(1)	3(1)				71(5)
increpitus	1(1)	33(2)		33(1)					67(4)
intrudens	40(2)				2(1)				42(3)
niphadopsis					1(1)				1(1)
pionips			1(1)						1(1)
punctor	42(3)	2(1)	12(1)		1(1)				57(6)
riparius	29(2)	27(1)	3(2)						59(5)
spencerii	6(2)		10(1)						16(3)
vexans			2(1)			126(4)			128(5)
Total	209(8)	233(5)	80(4)	160(2)	37(1)	569(6)	20(2)	163(2)	1471(30)

also indicates an aspect of seasonal fluctuations of mosquitoes.

From these tables and some other data, seasonal occurrence of mosquitoes in 1964 is given below.

Anopheles

Anopheles earlei Vargas hibernates as an adult female. Many larvae were found from late May to early July (Table 2), and one female was collected at the campus of the University of Alberta on May 26. Most of 58 larvae collected in late May shown in Table 2 were in the second instar and a few were in the first and a few in the third. Thus it seems that hibernated females appear and oviposit their eggs from May, and the emergence of adults occurs from June. Oviposition continued at least until the beginning of July, as two first instar larvae were encountered in early July.

Culex

The species of *Culex* found were *C. tarsalis* Coquillett and *C. territans* Walker. Both hibernate as adult females.

Although only one larva of *C. tarsalis* was collected, the hibernated females are considered to oviposit late in the season, as it is reported that in irrigated areas of Alberta the larvae are found abundantly in July, August, and September (Shemanchuk, 1959), and in Saskatchewan the first larvae do not appear until early July (Rempel, 1953).

The larvae of *C. territans* were collected in late June to late July (Table 2), and this seems to be also a late-appearing species.

Culiseta

Three species were encountered around Edmonton, namely

Table 3. The number of female mosquitoes collected around a pool on the south bank of the North Saskatchewan River near the University of Alberta.

Species	May		June				July				Aug.		Sept.		Total
	19	25	2	8	16	22	6	13	23	30	10	25	11	16	
Culiseta															
inornata		1	1	1	1	1		1							6
Aedes															
cataphylla	1														1
cinereus						1	1				1	3			6
communis		1		1	2	2									6
excrucians						1									1
fitchii						12	2	7	1				1		23
hexodontus		1		1		1									3
implicatus	4	3		1		25	1								34
increpitus						3	1			2	1				7
intrudens			1												1
punctor				1				2				1			4
riparius						1		2	1		1				5
stimulans														1	1
vexans								3			3				6
Total	5	6	2	5	3	47	5	15	2	2	6	4	1	1	104

One hour collection was made in the afternoon each day, excepting two hour collection on June 22.

C. alaskaensis (Ludlow), *C. inornata* (Williston), and *C. morsitans* (Theobald). They all hibernate as adult females.

The first egg raft of *C. alaskaensis* was found on July 6 (cf. Section 2.3) and one larva was collected in late July (Table 2). According to Jenkins (1948), overwintered females were common from late April to mid-June and all instars of larvae were found from May 11 to July 10 in Alaska. Therefore, the larvae may appear earlier than July also around Edmonton.

Table 2 indicates that the larvae of *C. inornata* were collected from early June, and this agrees with the data mentioned in Section 2.3. The peak of oviposition was found to be in early June and the peak emergence occurred in mid-July. The feeding activity seems to be limited mainly to the period from late May to early July, as judged from the number of females attracted to man (Table 3), and this is justified by the time of the peak of oviposition. Those females are considered overwintered ones. However, a small number of females oviposited as late as August 10 (cf. Section 2.3). It is not known whether such oviposition was derived from overwintered females or from newly emerged ones.

Two larvae of *C. morsitans* were obtained in early June. Rempel (1953) reported the adults in July. This species perhaps spends a similar life cycle to *C. alaskaensis* and *C. inornata* in Alberta.

Aedes

All *Aedes* species recorded here hibernate as the egg stage.

Black-legged species belonging to the subgenus *Ochlerotatus* are generally earlier-appearing species than other mosquitoes. The dates

of the collections of the larvae and adults in those black-legged species (from Tables 2 and 3), together with the records of the larvae and adults in Saskatchewan by Rempel (1953) and the dates of emergence near Edmonton by Klassen and Hocking (1964) are shown in Table 4.

It is apparent from the table that the larvae of most species appear very early in the season. However, *A. pionips* Dyar is perhaps a slightly later species, as indicated by Haufe (1952) and Rempel (1953), and it seems in *A. intrudens* and *A. punctor* that the hatching from eggs continues until later in the season, or the life span of the adults is longer. As for *A. niphadopsis* it is not clear, as only one larva was collected.

For banded-legged mosquitoes of subgenus *Ochlerotatus*, similar data are also given in Table 5.

Of the species given in the table, *A. excrucians* Walker, *A. fitchii* (Felt and Young), *A. increpitus* Dyar, *A. riparius* Dyar and Knab, and *A. stimulans* (Walker) are considered woodland species and have only one generation a year. The larvae appear as early as most black-legged mosquitoes, but the emergence is delayed because of slower development, as indicated by Haufe (1953 and 1956) and as recognized by the fact that the black-legged mosquitoes emerged earlier than the banded-legged ones, when the larvae from the same pool were reared in the laboratory. The females were collected as late as September 11 in *A. fitchii*, as August 10 in *A. increpitus*, and *A. riparius*, and as September 16 in *A. stimulans*. These facts seem to indicate that the life span of adults of those species is very long, as Rempel (1953) stated that occasional specimens of *A. excrucians* may be encountered in mid-summer.

Table 4. Summary of the occurrence of black-legged *Ochlerotatus*.

Aedes (Ochlerotatus)	Collections (1) of Larvae	Collections (1) of Adults	Records (2) of Larvae	Records (2) of Adults	Dates of emergence (3)
cataphylla	early Apr. -early May	May 19	late Apr.	early May	May 14 - June 15
communis	early Apr. -early May	May 25 -June 22		mid-May	May 30 - June 7
hexodontus	early Apr. -early May	May 25 -June 22			
impiger				late May	May 19
implicatus	early Apr. -early June	May 19 -July 6			May 14 -June 17
intrudens	early Apr. -early June	June 2		June 5 -Aug. 18	
niphadopsis	early June				
pionips	early May		as late as mid-July		
punctor	early Apr. -early June	June 8 -Aug. 25	May		
spencerii	early Apr.		common in late Apr.	abundant by May 10	

(1) Tables 2 and 3; (2) Rempel, 1953; (3) Klassen and Hocking, 1964.

Table 5. Summary of the occurrence of banded-legged *Ochlerotatus*

Aedes (*Ochlerotatus*)	Collections (1) of Larvae	Collections (1) of Adults	Records (2) of Larvae	Records (2) of adults	Dates of emergence (3)
campestris	late Apr.			(4) early June (5)	May 19
canadensis	late Apr.			mid-May - late July	May 30 -June 7
dorsalis	late May -late June		generally early July (4)	late July - Aug.	
excrucians	early Apr. -early June	June 22	early May	(5)	May 27 - June 4
fitchii	early Apr. -early June	June 22 -Sept. 11	May	June -early July	May 27 - June 17
flavescens	late May -early June		mid-May (4)	generally June-July	June 7 -June 17
increpitus	early Apr. -late May	June 22 -Aug. 10	early May	late May -June	
riparius	early Apr. -early May	June 22 -Aug. 10		mid-late May	
stimulans		Sept. 16	late May	late May -early July	May 30 - June 4

(1), (2) and (3) see Table 4; (4) a second generation may occur; (5) long-lived species, occasional specimens may be encountered late in the season.

A. canadensis (Theobald) is also a wood-loving species. The larvae appeared as early as other banded-legged species mentioned above. Occasionally hatching occurs in the fall in Illinois (Horsfall, 1955).

Other tabulated species, A. campestris Dyar and Knab, A. dorsalis (Meigen), and A. flavescens (Müller), are grassland-lovers, and a second generation may occur, when the environment is favorable. They seem to be slightly later-appearing species than the woodland species.

Aedes vexans (Meigen), which belongs to the subgenus Aedimorphus, is found in the three main ecological zones in Saskatchewan, the prairies, aspen grove region, and coniferous forest (Rempel, 1953). This species seems to have multiple generations when the conditions are favorable. The larvae were collected in early May to late June and the adults on July 13 and August 10. It is apparently a late-appearing species.

Black-legged A. (Aedes) cinereus (Meigen) seems to be rather late in appearance, though the first larva was collected in late April. The adults were collected from June 22 to August 25.

3. DISTRIBUTION PATTERN OF MOSQUITO LARVAE

3.1. Introduction

Populations of animals may be effectively estimated on the basis of their distribution pattern, and much has been published on this subject with various kinds of animals, among which, however, mosquitoes are not included. In applying the sequential sampling technique, which will be described later, and also in comparing the population densities at different pools, it is required to establish the nature of the frequency distribution

pattern of mosquito larvae (and pupae).

A dipper is usually used for collecting mosquito larvae, and is considered a handy and reliable tool. Here, an attempt has been made to analyse the distribution pattern of mosquito larvae in their habitats by using the number per dip.

3.2. Collections used for the determination of the frequency distribution

Table 6 gives the data of collections of mosquito larvae for determining the frequency distribution pattern of the numbers per dip. Collections numbers 9 to 24 in the table are the same data as used for the seasonal fluctuation of *C. inornata* described in Section 2.3. The table indicates that the collections were made at various habitats of various sizes during the period covering May 25 to September 30, and the mosquito species collected were distributed in the genera *Anopheles*, *Culex*, *Culiseta*, and *Aedes*. The habitats included a grassland pool, a woodland pool, a collection of scattered small pools, and the marginal part of a creek, and the mosquitoes were found at some times as a single species, and at others mixed.

3.3. The relation between mean and variance of the numbers per dip

In Table 7 the mean ($\bar{x}$), variance (s^2), and range of the numbers of mosquitoes per dip are given. The means vary from 0.02 to 39.40, and the variances from 0.02 to 3975.34. The minimum value of the range for most collections is zero, and the maximum value is up to 206. These figures indicate a great variability in number of mosquito larvae between the pools and also within each pool.

Table 6. Collections of mosquitoes in immature stages for determining the frequency distribution pattern.

Collection number	Date	Habitat	No. of dips	Mosquitoes collected
1	May 30	Permanent pool in open place	100	*Anoph. earlei*; *Aedes* spp.
2	May 30	Temporary grass-land pool	100	*Anoph. earlei*
3	June 7	Collection of small pools in pasture	100	*Culiseta* spp.; *Aedes* spp.
4	June 21	Permanent pool in open place	50	*Anoph. earlei*
5	June 24	Marginal part of a creek	60	*Anoph. earlei*; *Culex territans*; *Culiseta inornata*
6	June 24	Same as No. 3	30	*Culiseta inornata*; *Aedes* spp.
7	July 29	Same as No. 3	100	*Culex territans*; *Culiseta* spp.
8	July 29	Same as No. 5	40	*Culex* spp; *Culiseta inornata*
9 to 24	May 25 to Sept. 30	Permanent wood-land pool (See Section 2.3)	10-50	*Culiseta inornata*

Table 7. Mean, variance, and range of the numbers of mosquitoes per dip, together with x^2 - test for significant departure from Poisson distribution

Collection number	Mean (x)	Variance (s^2)	Range	x^2
1	1.61	5.47	0 - 11	336.60 **
2	0.56	0.89	0 - 4	157.41 **
3	0.49	2.76	0 - 15	557.37 **
4	0.02	0.02	0 - 1	49.00
5	0.20	0.82	0 - 5	161.07 **
6	14.60	509.21	0 - 85	1011.52 **
7	1.00	2.51	0 - 10	248.49 **
8	2.43	45.53	0 - 35	730.86 **
9	0.02	0.02	0 - 1	49.00
10	15.00	561.11	0 - 76	336.69 **
11	35.70	3975.34	0 - 206	1002.15 **
12	33.30	1552.54	0 - 159	885.78 **
13	32.40	882.04	0 - 82	244.98 **
14	28.60	867.82	4 - 80	273.06 **
15	39.40	2590.27	1 - 164	591.66 **
16	17.40	703.38	0 - 88	363.78 **
17	19.30	368.46	0 - 50	171.81 **
18	11.40	212.93	1 - 44	168.12 **
19	6.30	58.23	0 - 24	83.16 **
20	5.50	74.28	0 - 26	121.59 **
21	1.60	11.60	0 - 11	65.25 **
22	0.45	1.52	0 - 4	64.22 **
23	0.70	2.34	0 - 5	30.06 **
24	0.20	0.18	0 - 1	8.10

x^2 with n-1 degrees of freedom is calculated by $(n-1)s^2/\bar{x}$. For further explanation see text. For collection number see Table 6.

** Discrepancy from Poisson distribution is significant at 1% level.

Several mathematical models have been developed to describe the distribution pattern of animal counts. When the distribution is considered random, a Poisson distribution is often applied. In the Poisson distribution, the probability for a given positive integer x, P(x), is given by

$$P(x) = e^{-m} m^{x} / x! \quad \ldots \quad (1)$$

where m is the mean. It is a property of the Poisson distribution that the variance is equal to the mean, and the expression $\frac{\Sigma (x - \bar{x})^2}{\bar{x}}$ gives a good approximation to x^2 with (n - 1) degrees of freedom, where n is a sample size (Andrewartha, 1961).

It is apparent from Table 7 that the variation of variance is much greater than that of mean, and the Poisson distribution does not seem to fit the data excepting collection numbers 4, 9, and 24. To make sure, the values of $\frac{\Sigma (x - \bar{x})^2}{\bar{x}} = \frac{(n - 1)s^2}{\bar{x}}$ were calculated as the fifth column of Table 7, which were highly significant except for the above three collections. This shows that a random distribution - Poisson distribution - could not be rejected in the number of mosquitoes per dip, when the population density was as low as 0.20, and discrepancy from Poisson became greater with the increase of the mean, an aggregated type of distribution being indicated.

As stated by Waters (1959), there will be some field counts for which x^2 test will show no significant departure from either Poisson or an aggregated-type distribution such as negative binomial. It seems that non-significant values of x^2 in collections 4, 9 and 24 are attributable to the sparsity of the population and consequent low expectation of occurrence of mosquitoes in individual dips.

3.4. Goodness-of-fit to the Poisson and the negative binomial

Insect counts in the field are often fitted fairly well by a negative binomial distribution (Andrewartha, 1961; Anscombe, 1949, Bliss, 1953), which is one of the aggregated-type distributions. The frequency distribution of the negative binomial is given by expanding the expression $(q - p)^{-k}$, where $q - p = 1$, $p = m/k$, m is mean, and k is a positive exponent. As the variance of a negative binomial approaches the mean, or the over-dispersion decreases, $k \rightarrow \infty$ and $p \rightarrow 0$. Under these conditions it can be shown that the distribution converges to that for the Poisson (Fisher et al., 1943).

Goodness-of-fit to the Poisson and the negative binomial was tested (Tables 8 to 11) for the data with 100 dips, i.e. collection numbers 1, 2, 3, and 7.

Theoretical frequencies for the Poisson were calculated successively by the following formulae. The probability of observing zero count, $P(0)$, is

$$P(0) = e^{-m}, \quad \ldots\ldots\ldots\ldots \quad (2)$$

and the probability of observing $(x + 1)$, $P(x + 1)$, is

$$P(x + 1) = m\,P(x)/(x + 1), \quad \ldots\ldots\ldots\ldots \quad (3)$$

substituting sample mean, $\overline{x}$, for population mean, m. The theoretical frequency is obtained by multiplying each probability by the sample size, 100.

The formulae to be used for the theoretical values of the negative binomial (Bliss, 1953) are:

Table 8. Goodness-of-fit of Collection No. 1 to Poisson and negative binomial distributions.

No. of larvae per dip	Observed (O)		Frequency Hypothetical Poisson(P)	N. Binom. (N)	$\frac{(O-P)^2}{P}$	$\frac{(O-N)^2}{N}$
0	48		20.0	44.0	39.20	0.36
1	17		32.2	20.8	7.18	0.69
2	11		25.9	12.3	8.57	0.14
3	8		13.9	7.7	2.50	0.01
4	4	8				
5	4		7.4	8.3	0.05	0.01
6	3					
7	1					
8	1	8				
9	2		0.6	6.9	91.27	0.18
11+	1					
Total	100		100.0	100.0	148.77	1.39
				DF	4	3
					P<0.001	0.50<P< 0.75

Table 9. Goodness-of-fit of Collection No. 2 to Poisson and negative binomial distributions.

No. of larvae per dip	Frequency: Observed (O)	Hypothetical: Poisson(P)	Hypothetical: N. Binom. (N)	$\frac{(O-P)^2}{P}$	$\frac{(O-N)^2}{N}$
0	66	57.1	64.6	1.39	0.03
1	20	32.0	22.5	4.50	0.28
2	8	9	8.2	0.11	0.00
3	4 } 6				
4+	2 }	1.9	4.7	8.85	0.36
Total	100	100.0	100.0	14.85	0.67
			DF	2	1
				P< 0.001	0.25< P< 0.50

Table 10. Goodness-of-fit of Collection No. 3 to Poisson and negative binomial distributions

No. of larvae per dip	Frequency: Observed (O)	Frequency: Hypothetical: Poisson (P)	Frequency: Hypothetical: N. Binom. (N)	$\frac{(O-P)^2}{P}$	$\frac{(O-N)^2}{N}$
0	77	61.3	83.3	4.02	0.48
1	15	30.0	7.2	7.50	8.45
2	4	7.3	3.3	1.49	0.15
3	1				
4	2 } 4	1.4	6.2	4.83	0.78
15+	1				
Total	100	100.0	100.0	17.84	9.86
			DF	2	1
				$P<0.001$	$0.001<P<0.005$

Table 11. Goodness-of-fit of Collection No. 7 to Poisson and negative binomial distributions

No. of larvae per dip	Observed (O)		Hypothetical: Poisson (P)	Hypothetical: N. Binom. (N)		$\frac{(O-P)^2}{P}$	$\frac{(O-N)^2}{N}$
0	53		36.8	54.4		7.13	0.04
1	21		36.8	21.7		6.78	0.02
2	16		18.4	10.8		0.31	2.50
3	4	5	7.7	9.0		0.95	1.78
4	1						
5	3	5	0.3	4.1		5.39	0.20
6	1						
10+	1						
Total	100		100.0	100.0		20.56	4.54
					DF	3	2
						P< 0.001	0.10< P< 0.25

$$P(O) = (1 + m/k)^{-k} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

and

$$P(x + 1) = (x + k)mP(x) / (x + 1)(k + m) \quad \ldots\ldots\ldots\ldots \quad (5)$$

The constant k can be computed by a property of the negative binomial that the variance, σ^2, is equal to $(m + m^2/k)$, where m is mean, substituting again sample mean and variance, $\bar{x}$ and s^2, for m and σ^2.

In all of four examples shown in Tables 8 to 11, highly significant departure from the Poisson was demonstrated ($p < 0.001$), which indicates that the distributions can not be considered random. On the other hand, those distributions agree well with the negative binomial, except for collection 3, in which some discrepancy from the negative binomial is apparent. In this case, 15 larvae per dip were recorded once, which is a very high count compared with the others. This high count contributes larger variance, which in turn, yeilds rather small value of k responsible for the discrepancy. Generally speaking, the frequency distribution of the numbers of larvae per dip seems to agree with the negative binomial. The disagreement with the negative binomial in collection 3 may be attributable to sampling error.

3.5 Fitting the negative binomial distribution with a common k

Comparison between the means of two or more distributions are more direct and unequivocal if they have the same relative dispersion in terms of k, and two approaches to a common k were described by Bliss and Owen (1958). The first of them is a regression moment estimate applicable to the present data. The following calculation is based on

Bliss and Owen (1958).

Two statistics, x' and y' are computed from the mean and variance of each component distribution:

$$x' = \bar{x}^2 - s^2/n \quad \ldots \quad (6)$$

$$y' = s^2 - \bar{x} \quad \ldots \quad (7)$$

where n is sample size. Their expectations are given exactly by

$$E(x') = m^2 \quad \ldots \quad (8)$$

$$E(y') = m^2/k \quad \ldots \quad (9)$$

Thus $(y' - x'/k)$ has zero expectation. For a single sample, we have the ratio

$$1/k_1 = y'/x' \quad \ldots \quad (10)$$

as an estimate of $1/k$. The variance of $(y' - x'/k)$ is given to order $1/n^2$ by

$$V = 2m^2 (m-k)^2 [k(k-1) - (2k-1)/n - 3/n^2] / (n-1)k^4 \quad \ldots \quad (11)$$

The invariance $w = 1/V$ is of the nature of a weight. If calculated by replacing m by $\bar{x}$, m^2 by x', and k by an empirical trial value of k', we can obtain an estimate of $1/k$, $1/k_c$, by

$$1/k_c = \Sigma(wx'y') / \Sigma(wx'^2) \quad \ldots \quad (12)$$

as the slope of a linear regression of y' on x', the regression line being constrained to pass through the origin ($x' = 0$, $y' = 0$).

Referring back to the data of Table 7, x' and y' were calculated by formulae (6) and (7) for each collection, and the relation between them is given in Fig. 2, in log scales so as to show the values with great variabilities in one chart.

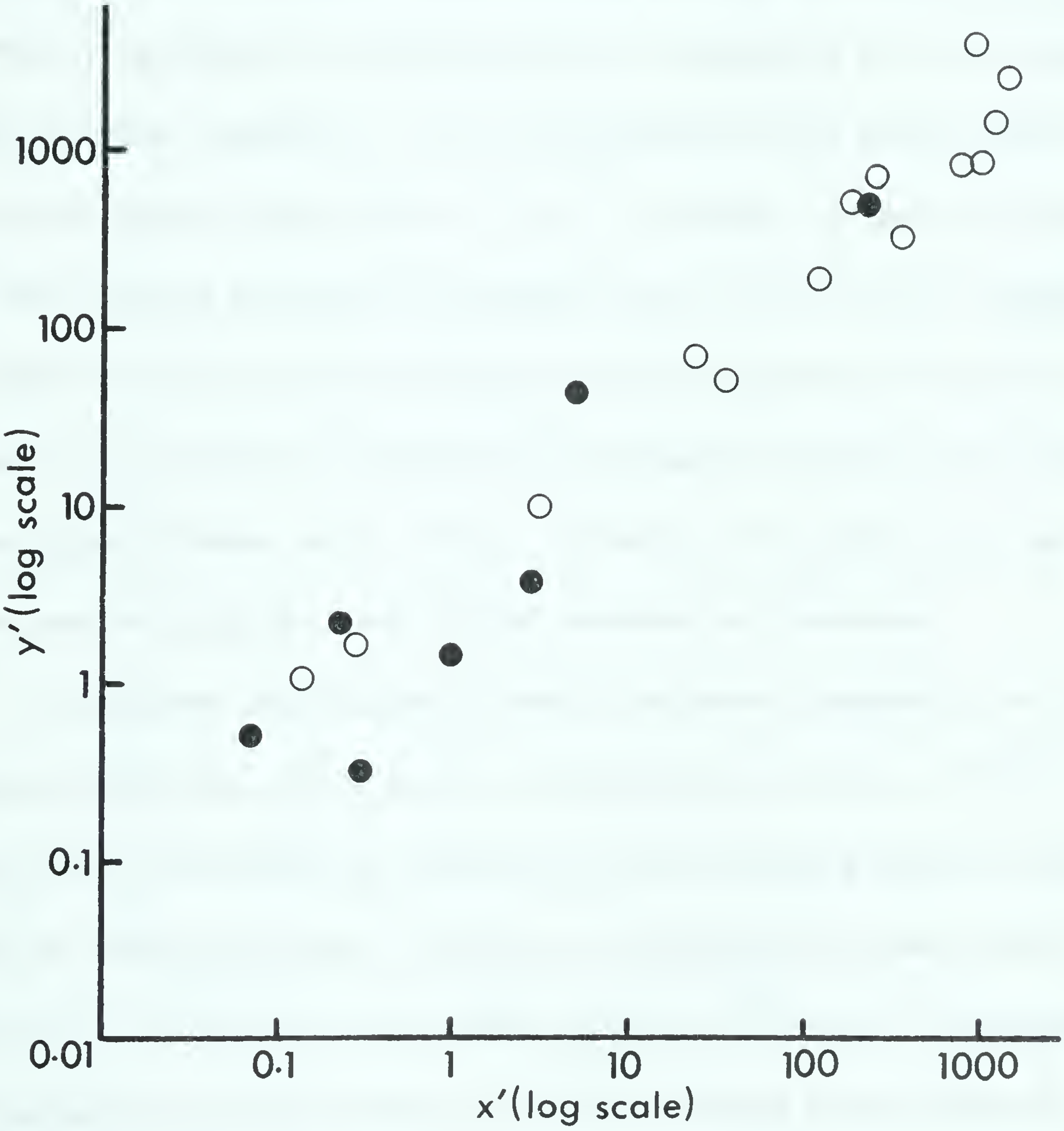

Fig. 2. Relation between two statistics, x' and y', defined by equations (6) and (7). ● : Collection Nos. 1 - 8; ○ : Collection Nos. 9 - 24. Collection Nos. 4 and 9 are not shown in the figure, because x' = 0, y' = 0, and also will be excluded in the later calculations, because of indeterminate values of y'/x'.

Assumed that a proportional relation holds between the two, that is given by $y' = (1/k)x'$, then the relation is represented by a straight line with an inclination of one in the figure in log scales, because $\log y' = \log (1/k) + \log x'$. The data of Fig. 2 satisfies the above assumption very well. This indicates that the relation between x' and y' is represented by a regression line passing through the origin, and, in turn the underlying frequency distributions are suggested to be the negative binomial with a common k. It is interesting that the same trend seems to be shown in the regression of y' on x' between collection numbers 1 to 8 for various species of mosquitoes and 9 to 24 for _C. inornata_ (see Table 6), because the inclination of the regression line gives the estimate of k, which is considered an intrinsic property of the population sampled (Fisher et al., 1943). However, it is likely that the value of k is species specific, and further studies are required.

It is known that in some cases k increases somewhat as m increases (Anscombe, 1949; Morris, 1954; Bliss and Owen, 1958). So, the values of $1/k_1$ calculated by equation (10) were plotted against mean, $\bar{x}$, in Fig. 3, which indicates, however, no appreciable relationship between the two. In order to know the exact situation, however, the number of dips for each collection seems to have not always been sufficient, and further investigations are required.

Now, a common value of k will be estimated. The statistics x' and y' for each of the distributions have already been obtained. The next step is to get an initial trial estimate of a common k, k'. As $\bar{x}$

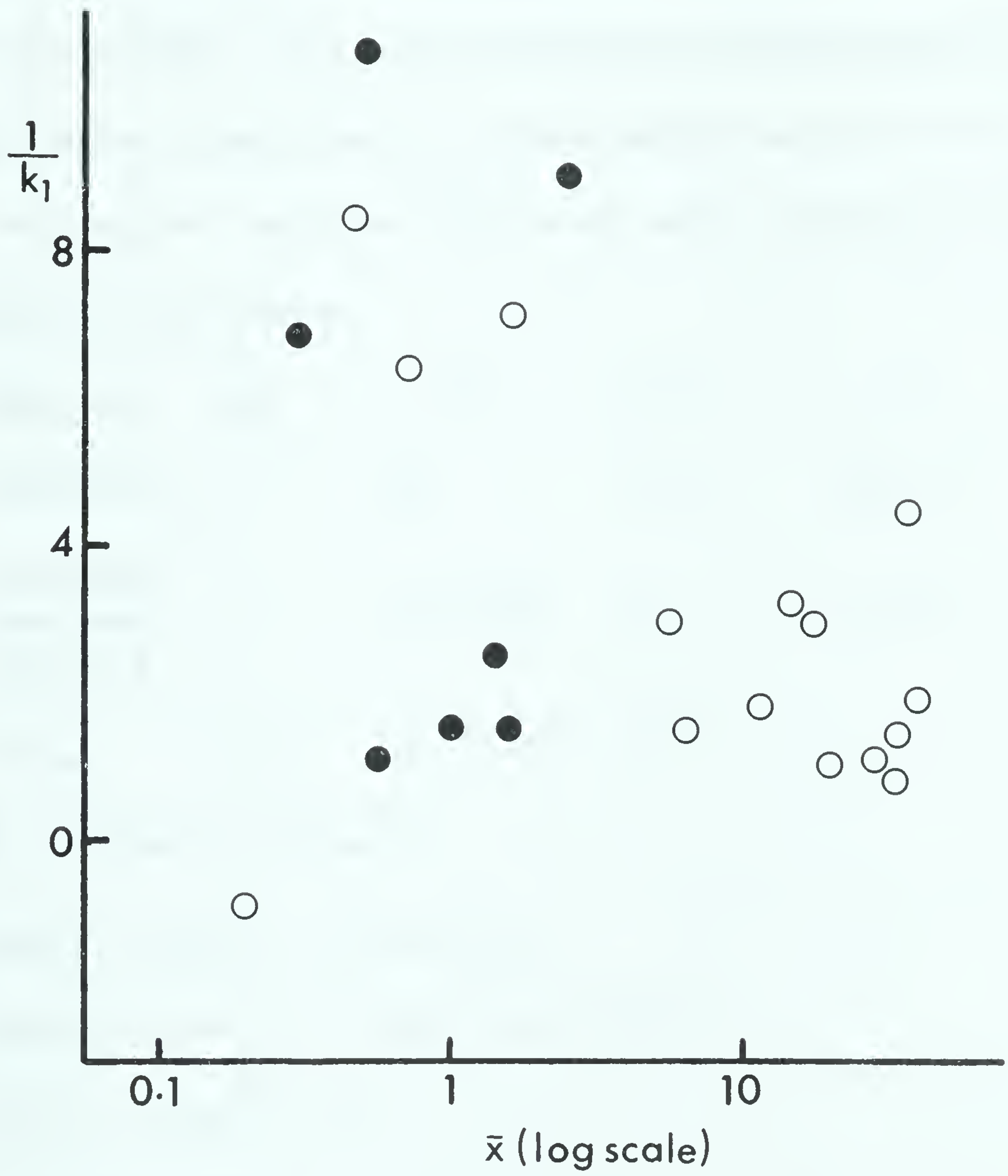

Fig. 3. Relation between mean ($\bar{x}$) and estimate of $1/k$ ($1/k_1$). ● : Collection Nos. 1 - 8; ○ : Collection Nos. 9 - 24.

varies excessively among the collections, a suitable equation for k' is

$$k' = g/\Sigma\,(y'/x') \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (13)$$

where g is the number of collections. Thus we got $k' = 0.2822$. By using this value, $1/k_c$, an estimate of $1/k$, was obtained by equation (12) and as its reciprocal $k_c = 0.2947$, which does not differ so much from the first trial estimate $k' = 0.2822$. Thus we have estimated a common value of k at 0.2947. If k_c should differ appreciably from its trial value, k', recalculation is necessary by replacing the initial k' by k_c.

The required tests for agreement with a single k_c may be arranged as an analysis of variance:

Effect of	DF	SS	MS	F
Slope, $1/k_c$	1	B_o^2	B_o^2	B_o^2 / S^2
Computed intercept against 0	1	$C+B^2-B_o^2$	I_o	I_o/S^2
Error	g-3	$[wy'^2]-B^2$	S^2	

where $B_o^2 = \Sigma^2(wx'y')/\Sigma(wx'^2)$

$$[wx'^2] = \Sigma(wx'^2) - \Sigma^2(wx')/\Sigma w,$$

$$[wx'y'] = \Sigma(wx'y') - \Sigma(wx')\Sigma(wy')/\Sigma w,$$

$$[wy'^2] = \Sigma(wy'^2) - C,$$

$$C = \Sigma^2(wy')/\Sigma w,$$

$$B^2 = [wx'y']^2/[wx'^2],$$

$$\Sigma^2\,(\text{—}) = (\Sigma\ (\text{——}))^2.$$

If a single k_c is justified, the F-value in the first row should be clearly significant and that in the second row not significant. The

calculated values are shown below:

Effect of	DF	SS	MS	F
Slope, $1/k_c$	1	33.7809	33.7809	24.4670**
Computed intercept against 0	1	4.0297	4.0297	2.9186
Error	19	26.2327	1.3807	

The results are highly significant for slope and not significant for computed intercept against 0, that is a common value of k is justified.

3.6 Consideration of reasons for a negative binomial distribution

I have demonstrated that the number of mosquitoes per dip follows a negative binomial distribution with a common k. The negative binomial is generated by a distribution that is "contagious" in the sense that the presence of one individual in a division increases the chance of other individuals falling into that division. However, as Andrewartha (1961) stated, agreement with the negative binomial does not itself permit any inference about the biology of the mosquitoes, though a significant discrepancy from the Poisson series disproves the hypothesis of random scatter. In fact, according to Bliss (1953) the negative binomial may be regarded as being compounded from a number of Poisson series in which the means vary in such a way that they are distributed like x^2, and furthermore it is possible to imagine a number of other models to explain it.

The present data are not considered the sum of a number of Poisson series with different means, and other reasons should be sought.

One of them which might arise is a dipping error, however, its effect seems to be of little importance, or at least, the negative binomial distribution is not attributable only to it.

No habitat of mosquitoes in nature is considered so uniform that all parts of it are equally attractive to them. Marginal parts of a pool are usually preferable to mosquito larvae, and it is a common phenomenon that the spatial distribution of the mosquitoes is related to water-plants or overgrown vegetation. Thus the heterogeneity of the environment seems to be a great reason for the contagious distribution - the negative binomial. In fact, Hocking (1953) observed strong aggregation of the larvae of Aedes communis DeGeer, due apparently to the effect of sunlight and temperature gradient in the pool.

Another reason to be considered here may be a gregarious habit of mosquitoes. Although this has not been studied extensively, it seems important in the ecology of mosquitoes. It is commonly observed in the laboratory that mosquito larvae show some aggregated distribution in a tray, in which the environment does not appear to differ appreciably. This habit of aggregation differs in intensity with species, and, for example, strong aggregation of larvae is frequently seen in Aedes aegypti (L.), but it is hardly ever seen in Anopheles hyrcanus sinensis Wiedeman. The biological meaning of this is not clear at the present time, but it is interesting in that it may be related to the level of optimum density of larvae. At any rate, the intrinsic behaviour of mosquitoes may play some role in the contagious distribution.

In short, the heterogeneity of habitat and possibly a sort of gregarious behaviour of mosquito larvae are considered to be responsible for the negative binomial distribution which is characterized by a larger variance than mean.

4. SEQUENTIAL SAMPLING TECHNIQUE

4.1. Introduction

Sequential sampling can be used for classifying a population into one of a number of pre-defined density levels, based on the accumulated results of each unit sampled. In classifying animal populations, it has been applied to the spruce budworm (Morris, 1954), whitefish, *Coregonus clupeaformis* (Mitchell) (Oakland, 1950), the lodgepole needle miner (Stark, 1952), and an aphid, *Myzus persicae* (Sulzer) (Sylvester and Cox, 1961). However, it has never been applied to mosquitoes.

The great value of this procedure lies in the fact that it involves a flexible sample size in contrast to conventional sampling procedures, and it would frequently be possible to determine whether or not a mosquito population requires control, or satisfactory control has been obtained, with the expenditure of much less time than would have been required if the number of sampling units was inflexibly fixed (Knight, 1964). Therefore, it would be reasonable to extend this technique to the immature stages of mosquitoes.

The procedure given by Morris (1954) is mainly followed by the present application.

4.2. Density classes

As mentioned above, the sequential sampling technique is used for classifying a population into pre-defined density levels. It is desirable that density classes are determined so as to enable us to know from these classes whether or not the mosquito density is so high that control operations are necessary, or whether a control operation has been successful.

The density classes may be differently set up according to the situation in the city or town concerned. Here, I have classified density tentatively into three levels indicated by the critical mean number of larvae per dip as follows:

Density	Mean No. of larvae per dip
Low	0.1 or less
Moderate	Between 0.5 and 2.5
High	12.5 or more

Density class "high" may be regarded as an indication that the mosquito density is so high that control is required, or that a control operation has influenced the population but little, and "low" may indicate that the density is so low that control is not required, or that control was satisfactorily done. "Moderate" is the intermediate situation between the two. Although the density is not so high control may be desirable if it is early in the mosquito season.

Of course, the necessity of controlling mosquitoes depends not only on the mosquito density in each habitat, but also on the relative area of the habitat compared with the whole area, as well as the location of

those habitats in relation to city or town to be protected from mosquitoes. However, it is still true that population density must be determined at each habitat before a decision to control or not to control is taken.

4.3. Acceptance and rejection lines

To apply the sequential sampling technique to the mosquitoes, of which number per dip is considered to follow the negative binomial distribution, it is necessary to find a common value of $\underline{k}$ fitting all the data with different levels of mean, and it has been determined as 0.2947 (see Section 3).

The next step is to set up alternative hypotheses, H_0 and H_1, from the density classes. To distinguish between low and moderate densities at a certain probability level, H_0 and H_1 are that the number of larvae per dip is 0.1 or less and 0.5 or more, respectively; to distinguish moderate and high they are that the number is 2.5 or less and 12.5 or more. The values of the constants based on the negative binomial distribution at the critical densities under these hypotheses are shown in Table 12.

Each pair of hypotheses is accompanied by two possible errors: α and β are the probabilities of rejecting H_0 and H_1 at the respective critical densities. Here, both α and β were set at 0.10. A rather large value for error probability seems to be suitable for rapid mosquito survey, because it reduces the number of dips to be taken at each habitat and enables us to decide whether or not control is necessary by a quick evaluation of the population density over a wide area in a relatively short time.

Table 12. Values of the constants at the critical densities under the hypotheses of H_0 and H_1, based on the negative binomial distribution.

	Density			
	Low - Moderate		Moderate - High	
Constant	H_0	H_1	H_0	H_1
Mean = kp	0.1	0.5	2.5	12.5
p = kp/k	0.3393	1.6967	8.4833	42.4163
q = 1 + p	1.3393	2.6967	9.4833	43.4163
Variance = kpq	0.1339	1.3484	23.7083	542.7038

Formulae for the acceptance and rejection lines then are:

$$d = sn + h_0 \quad \ldots \ldots \quad (13)$$

and

$$d = sn + h_1 \quad \ldots \ldots \quad (14)$$

where d is the cumulative number of larvae in the first n dips. The slope of the lines, s, is

$$s = k \log (q_1/q_0)/\log (p_1q_0/p_0q_1) \quad \ldots \ldots \quad (15)$$

where q_0 and q_1 are the values of q and p_0 and p_1 are those of p under the hypotheses of H_0 and H_1 (for actual figures see Table 12), and the intercepts of the equations (13) and (14) on the d-axis are

$$h_0 = \log B/ \log (p_1q_0/p_0q_1) \quad \ldots \ldots \quad (16)$$

where $B = \beta\ /(1-\alpha\ )$ $\quad \ldots \ldots \quad$ (17)

and

$$h_1 = \log A/ \log (p_1q_0/p_0q_1) \quad \ldots \ldots \quad (18)$$

where $A = (1-\beta\ )/\alpha$ $\quad \ldots \ldots \quad$ (19)

Thus we get the following formulae as acceptance and rejection lines for low versus moderate classes,

$$d = 0.2267n - 2.4153$$

and

$$d = 0.2267n + 2.4153,$$

and for moderate versus high

$$d = 5.0891n - 24.9138$$

and

$$d = 5.0891n + 24.9138,$$

as shown in Fig. 4. This graph may be used in the field to determine how

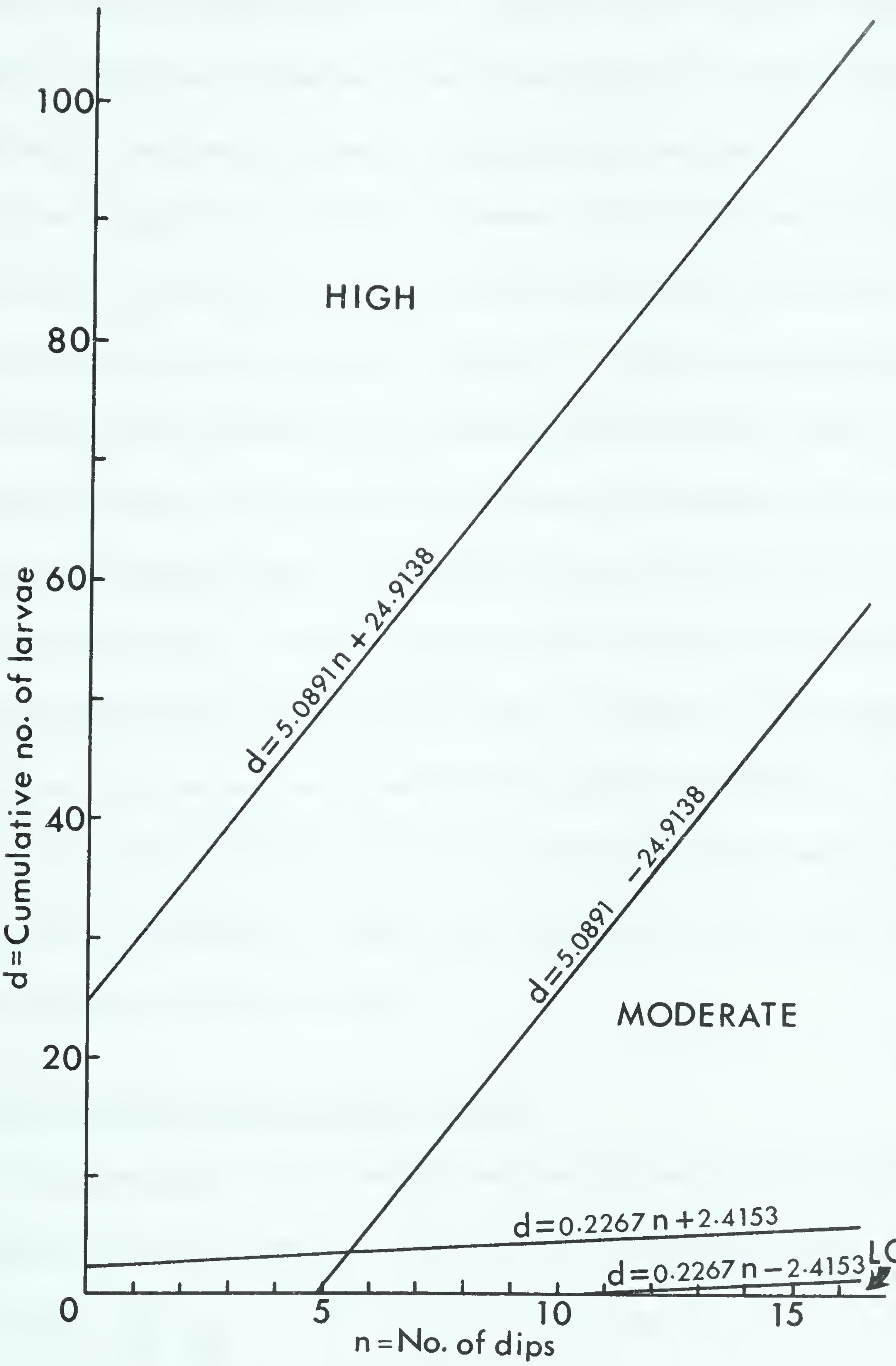

Fig. 4. The acceptance and rejection lines.

many dips should be taken at each habitat in order to define the density class within the accepted limits of α and β. It is helpful to visualize each pair of lines as enclosing a band from which the plotted points must escape before the density class is satisfactorily defined.

For example, in collection number 1 mentioned earlier (see Tables 6 and 7 of Section 3), the first three dips show no larvae. When zero is plotted over each number of dips 1, 2, and 3, it is seen that they are within both bands of low-moderate and moderate-high. The fourth dip yields two larvae and the fifth none, therefore dips 4 and 5 are still within these bands. The sixth dip shows three larvae, so 2 + 3 = 5 is plotted over dip 6. This is shown to have escaped from the bands and to have fallen into the moderate zone, so dipping is discontinued. Thus collection number 1 is classified into moderate density. If the plotted points had escaped into the area above the higher band, the density would be classed as high, and if below the lower band, the density would be classed as low.

4.4. The operating characteristic curves

The operating characteristic curves are useful aids in understanding how the plan operates. The curve is calculated from

$$L(p) = \frac{A^h - 1}{A^h - B^h} \qquad (20)$$

$$p = \frac{1 - (q_0/q_1)^h}{(p_1 q_0/p_0 q_1)^h - 1} \qquad (21)$$

where $L(p)$ is the probability of accepting H_0 for any possible level of the

population mean of kp, A and B are taken from equations (19) and (17), and h is a "dummy variable" which may be assigned convenient values.

The operating characteristic curve is shown in Fig. 5 by plotting L(p) against population mean, kp. The left-hand curve is for low versus moderate density classes. When the mean, kp, is 0.1, the probability of accepting H_0 (low density class) is 0.9; accordingly the probability of accepting H_1 (moderate density class) is 0.1. When kp = 0.5, L(p) = 0.1 for H_0 and consequently 0.9 for H_1. At these two levels of kp, the probabilities correspond, of course, to those previously set for α and β. As kp decreases below 0.1, L(p) for H_0 becomes very high, and as it increases above 0.5, L(p) for H_0 becomes very low. When kp is ca. 0.23, the chances of accepting H_0 and H_1 are equal. The curve on the right is used in the same way for the moderate versus high density classes. The overlapping between the two curves is only at negligible probability levels. Thus the probability of considering a low density class high, or high density class low, is very small.

4.5. <u>The average sample number curves</u>

The average sample number curves can be drawn by plotting the values for E(n), the mean number of dips which must be taken, against kp, the mean number of larvae per dip, as shown in Fig. 6. For different values of kp, E(n) is calculated from

$$E(n) = \frac{h_1 - (h_0 - h_1)\,L(p)}{kp - s} \quad \ldots\ldots\ldots\ldots \quad (22)$$

where h_0, h_1, L(p), and s are taken from equations (16), (18), (20), and

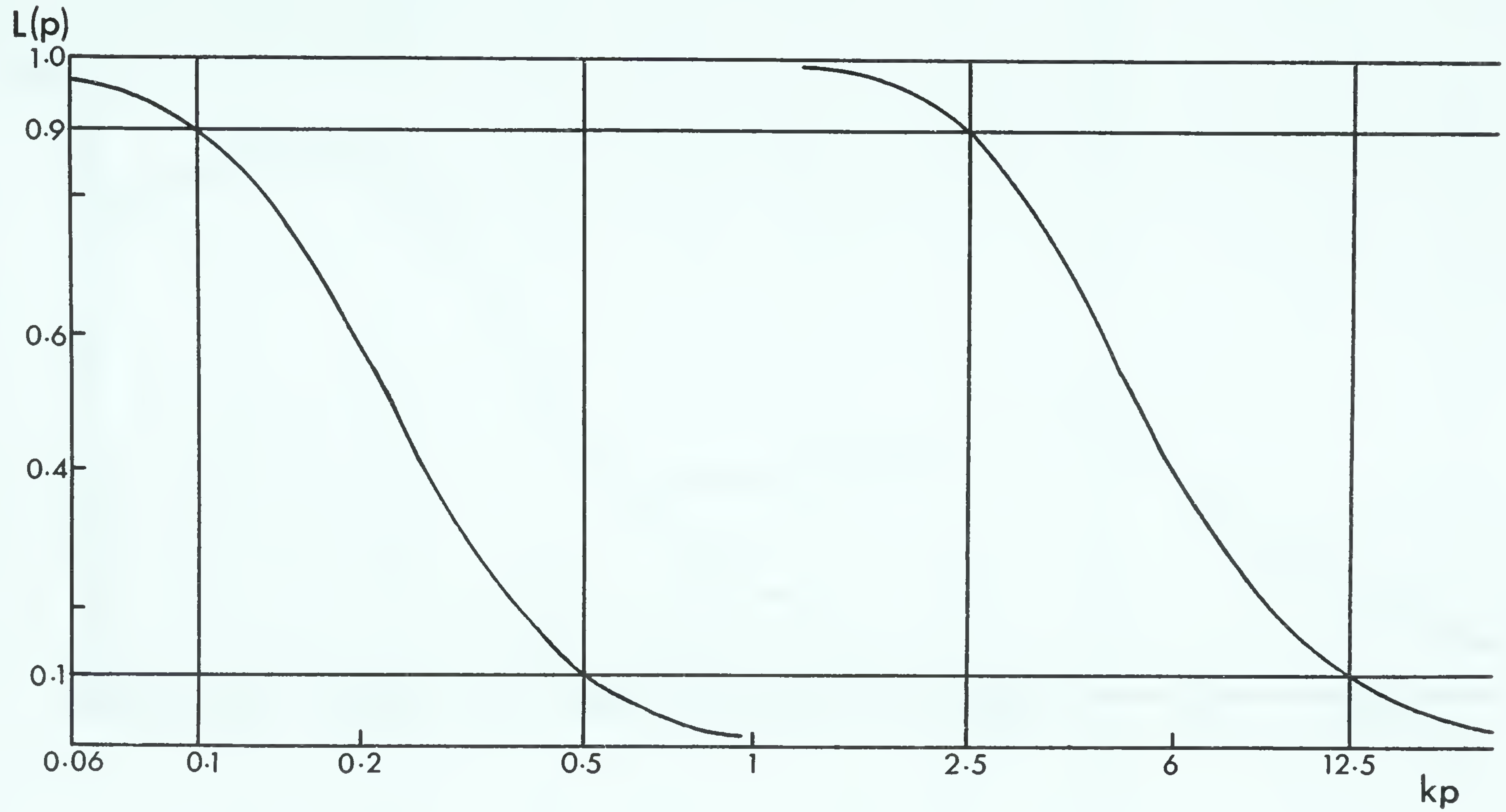

Fig. 5. The operating characteristic curves for low versus moderate density classes (left) and for moderate versus high (right). kp = mean no. of larvae per dip; L (p) = probability of accepting H_O hypothesis.

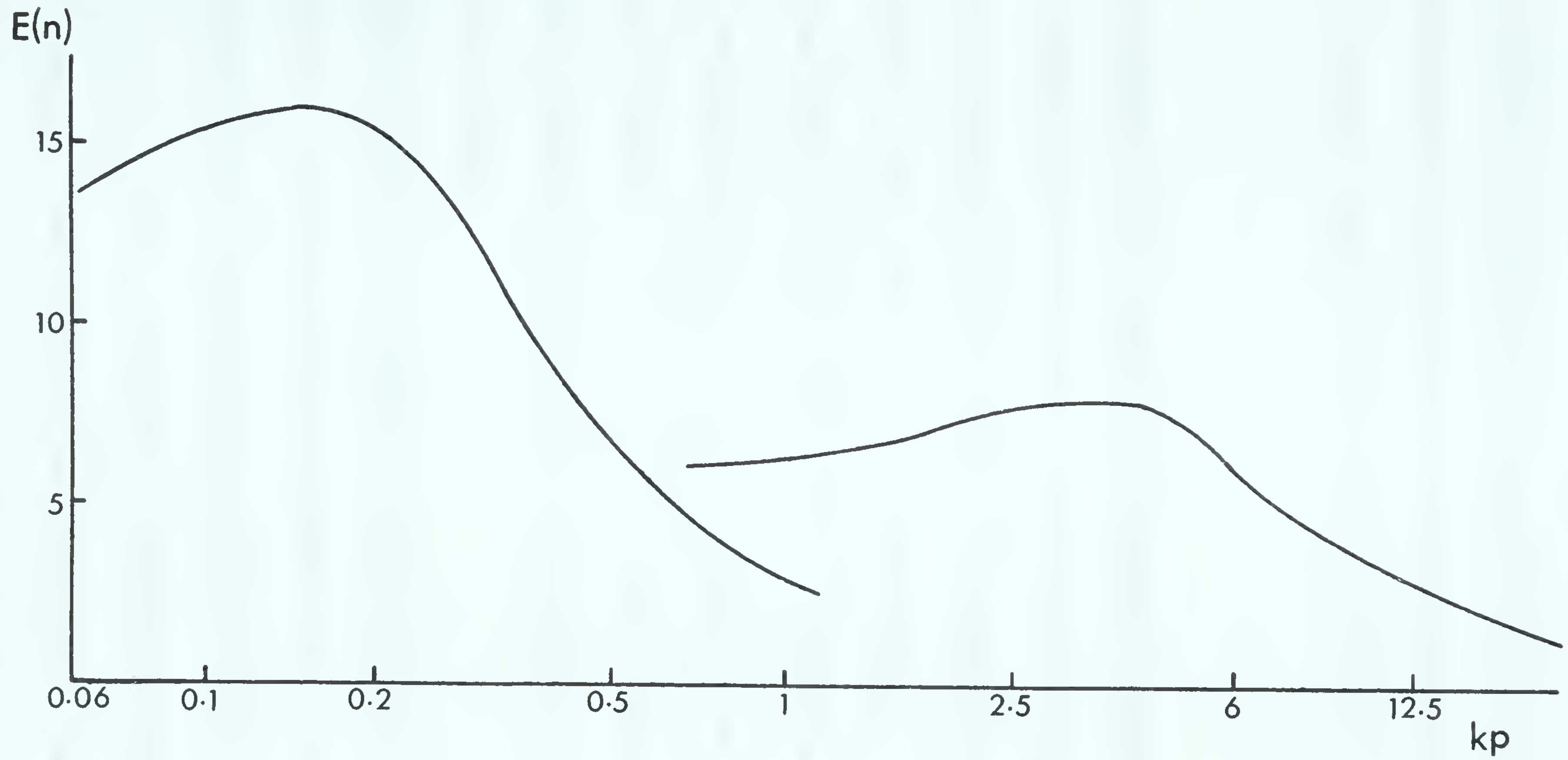

Fig. 6. The average sample number curves for low versus moderate density classes (left) and for moderate and high (right). kp = mean no. of larvae per dip: E (n) = mean no. of dips to be taken.

(15), respectively. $E(n)$ does not indicate the number of dips which must be taken actually at each pool, but its expectation.

As would be expected, the peaks of the curves in Fig. 6 occur where populations are borderline between low and moderate or between moderate and high, which indicates that relatively more dips are required there.

4.6. Applications of the sequential sampling technique in the field

In applying the sequential sampling technique in the field it is convenient to use tabulations (Table 13) prepared from the acceptance and rejection lines, rather than the lines themselves. Dipping is continued until the cumulative number falls into one of the density classes. It is apparent from the table that at least 11 dips are necessary for the density to be classed into low, and at least six into moderate; if the number of larvae in the first dip is 31 or more, the density is classified as high without further dips.

Table 14 gives the results of applications of the sequential sampling technique to the data shown in Tables 6 and 7 of Section 3. It is demonstrated that the sequential plan can be used to classify the density correctly into one of low, moderate, and high density classes. The number of dips required for determining the class in various collections ranged from 1 to 20. When the density is high, the required number of dips was rather small, as expected from Fig. 6. This is of advantage in field work, because it takes much more time to count larvae dipped when the density is higher.

Table 13. Sequential table for use by field parties, prepared from the acceptance and rejection lines (Fig. 4).

No. of dips	Cumulative No. of larvae		
	Low	Moderate	High
1			31 or more
2			36 or more
3			41 or more
4			46 or more
5			51 or more
6		4 to 5	56 or more
7		5 to 10	61 or more
8		5 to 15	66 or more
9		5 to 20	71 or more
10		5 to 25	76 or more
11	0	5 to 31	81 or more
12	0	6 to 36	86 or more
13	0	6 to 41	92 or more
14	0	6 to 46	97 or more
15	0	6 to 51	102 or more
16	0 to 1	7 to 56	107 or more
17	0 to 1	7 to 61	112 or more
18	0 to 1	7 to 66	117 or more
19	0 to 1	7 to 71	122 or more
20	0 to 2	7 to 76	127 or more

Continue to dip until the cumulative number falls into one of the 3 density classes of low, moderate and high.

Table 14. Application of the sequential sampling technique to the data shown in Tables 6 and 7 of Section 3.

Collection number	Mean No. of larvae	Density class determined	No. of dips required
1	1.61	Moderate	6
2	0.56	Moderate	20
3	0.49	Low	11
4	0.02	Low	16
5	0.30	Moderate	18
6	14.60	High	9
7	1.00	Moderate	20
8	2.43	Moderate	7
9	0.02	Low	11
10	15.00	High	1
11	35.70	High	2
12	33.30	High	2
13	32.40	High	2
14	28.60	High	1
15	39.40	High	1
16	17.40	High	7
17	19.30	High	3
18	11.40	High	3
19	6.30	High	2
20	5.50	Undetemined*	⩾11
21	1.60	Moderate	9
22	0.45	Moderate	6
23	0.70	Moderate	7
24	0.20	Undetermined*	⩾11

* During 10 dips made, the density class was not determined.

In sampling, the larvae are required to be dipped all over a pool. In a large pool, dividing it into a few portions and applying the sequential plan at each will facilitate the work. Suggested larval survey form is given in Table 15.

This technique can be used effectively for the evaluation of the application of larvicides in a relatively short time. If the control operation is successful, then the densities at all pools will fall into the low density level. Also, this may be used for determining whether or not a second larvicide application is required specifically for the later-appearing mosquitoes. Necessity for mosquito control depends on the productivity of mosquitoes in a particular area, rather than the population density at each pool. To approach this, the following procedures may be appropriate. Firstly we determine the density class at each pool by the sequential sampling technique. Then, we take 0, 1, and 10 as indices for low, moderate, and high density levels, respectively, and multiply the index by the area of the pool (the area of the marginal parts if the larval distribution is confined there). If these are summed for a district to be examined, then it will represent the productivity of mosquito there. The sequential plan may be used for comparing regional differences of mosquito abundance, which provide us with the knowledge as to which region should be stressed for larval control operations.

5. SUGGESTED STUDIES TOWARD BETTER CONTROL OF EDMONTON MOSQUITOES

5.1. Introduction

In this section, only ecological questions are discussed, although

Table 15. Suggested mosquito larval survey form for the application of sequential sampling technique in the field.

MOSQUITO LARVAL SURVEY FORM

Collection No. Collector:

Place: Hour: , a.m. p.m.

Date: , 19

Breeding place
- -type: woodland-pool, grassland-pool, roadside-ditch, small pools in pasture, creek, other ( )
- -permanent, temporary
- -size
- -notes (marginal vegetation; water plants; animals; temperature, pH, cleanness of water; etc.)

No. of dips	No. of larvae	Cumulative No.	No. of dips	No. of larvae	Cumulative No.
1			11		
2			12		
3			13		
4			14		
5			15		
6			16		
7			17		
8			18		
9			19		
10			20		

Density class determined: Low, Moderate, High
Instar of larvae:
Species identified:

studies are also needed on the identification of mosquitoes including the larvae in younger instars, the development of insecticidal resistance, the methods and evaluation of applications of chemicals, the effective and economical dosages of larvicides and adulticides, the residual effects of insecticides when applied to the habitat in the field, and so on.

5.2. The time of hatching and emergence

The prediction of the emergence time of mosquitoes is required to determine the appropriate time for chemical control. The best time for controlling mosquito larvae is before they begin to pupate, the pupae being much more resistant to insecticides than larvae, but not before hatching is complete. Strictly speaking, the above situation is hard to realize in the field, because the time of hatching differs between species and also within species so that there remain some eggs of *Aedes* to be hatched later in the season after some adults have emerged. Thus the most effective time for insecticidal applications against mosquito larvae is our special concern. For this purpose, many points remain to be studied. These include the studies on the time of oviposition and the durations of egg and larval stages.

In mosquitoes belonging to the genera *Anopheles*, *Culex*, and *Culiseta*, which overwinter as adults, the time of oviposition depends on the time of blood feeding and the duration of egg development. Blood feeding is certainly related to temperature and possibly to adult diapause. The temperature apparently influences the maturation of eggs.

All *Aedes* mosquitoes found around Edmonton overwinter as eggs.

According to Clements (1963), the different *Aedes* species fall fairly clearly into those whose eggs enter diapause and require reactivation, and those whose eggs merely become quiescent and hatch shortly after exposure to an adequate hatching stimulus, although they may require a few hours conditioning. Obligatory diapause in the egg stage is found in *Aedes hexodontus* (Beckel, 1958), in *Aedes squamiger* (Telford, 1958), and in *Aedes stimulans* (Horsfall and Fowler, 1961), where exposure to low temperature is required before egg diapause can be broken. These mosquitoes have only one generation a year. Multivoltine species have facultative diapause, as in *Aedes dorsalis* (Khelvin, 1958), *Aedes nigromaculis* (Telford, 1963), and *Aedes triseriatus* (Baker, 1935), or have no diapause.

Most mosquitoes found around Edmonton have one generation a year. However, there is a possibility that a second generation occurs in some species, such as *A. campestris*, *A. dorsalis*, or *A. flavescens*, perhaps in August when the conditions are favorable.

It is very likely that there is a wide variability in hatching response of eggs, so that the time of hatching has a wide range, even for eggs from the same batch.

Beckel (1958), Telford (1963) and others have discussed the mechanism and ecological significance of egg diapause in mosquitoes, and much has been published on the hatching response in quiescent eggs of *Aedes aegypti* and some other *Aedes* species (see Telford, 1963). However, the situation is still not clear for most *Aedes* mosquitoes.

After hatching from eggs, the development of larvae depends on

various factors. The most important are temperature, quality and quantity of food, and larval density. It is expected that the relation between larval period and temperature is described by an equilateral hyperbola, or the relation between developmental speed and effective temperature (temperature minus developmental zero point) is linear, at least within a reasonable temperature range, provided that other factors than temperature are constant. Based on this relation, Haufe (1953) and Haufe and Burgess (1956) attempted to predict dates of emergence in mosquitoes at Fort Churchill, Manitoba, and stated: "The tundra species of mosquito (*A. impiger* and *A. nigripes*) had lower thresholds of development approximating 34 F; the forest species (*A. communis*, *A. punctor*, *A. excrucians*) had a range of 38 - 40 F, except *A. hexodontus*. The products of time and temperature for the period of development of both tundra and forest species were lower for the smaller than for the larger species". Studies of this sort are desirable for all the mosquito species found abundantly around Edmonton.

It is to be noted that the threshold of development obtained from the above relation is slightly higher than the actual value in the development of most insects, and is not necessarily the same as the critical temperature for hatching. Also, the developmental speed differs greatly according to factors such as quality and quantity of food and larval density. Thus, for the prediction of the date of emergence, careful investigations are required in the laboratory for each species. Another aspect to be involved is the relation between temperature in pools in various situations and meteorological records, for example see Haufe and Burgess (1956) and Haufe (1957).

5.3. Flight range

Southwood (1962) stated: "It is suggested that animal movements fall basically into two types: trivial and migratory. Trivial movements are normally confined to the territory or habitat of the population to which the animal belongs, migratory movements carry the animal away from this area. Although there is undoubtedly no sharp line but a gradation between these two types, they can be distinguished by various ecological, physiological and behavioural characteristics", and "The ideal evidence of migratory movement is that while engaged in it the animal does not respond to food, a mate or habitat, and moves from the actual territory where it has developed into an inhospitable terrain; such movement is normally at the start of adult life". Provost (1957) reported the findings of a mark-and-release experiment with Aedes taeniorhynchus as follows: "Migration occurs the night of departure only, therefore twilight departures will result in longer migrations than middle-of-the-night departures. Appentential (trivial of Southwood, 1962) flights expand the range of occupation by a brood much beyond what is established by the migration." Thus mosquito dispersal consists of two phases of movement, and its range depends greatly on the migratory flight and to a lesser extent on the appetential flight.

In the appetential flight of mosquitoes, the distribution of breeding, resting, feeding, and oviposition sites, and in some species overwintering sites, will influence the degree of dispersal, because in mosquitoes these sites are situated quite often at different places and it is suggested that, within limits, the closer these are situated, the shorter the flight range.

This should be considered in the field data, particularly when mark-and-release experiments are conducted.

As mentioned in Section 2, 14 species of adult female mosquitoes were collected around a pool near the University of Alberta. Excepting Culiseta inornata, all these mosquitoes are considered to have entered from outside or marginal parts of the City of Edmonton, since there are no breeding places for these species in the central part. This means that they dispersed at least a few miles, and should be considered potential pests for the Edmonton area according to their abundance. Here, it is required to determine the range of dispersal for each species. It is not known whether migratory flight was involved in the dispersal or not. It seems reasonable to suppose that the range of dispersal is longer in mosquitoes where migratory flight is involved than in others. The investigation of this subject will help decide how widely insecticidal application or other control of larvae must be extended to control mosquitoes near Edmonton.

5.4. Fluctuation in numbers

The habitats of most mosquito larvae are characterized by their unstableness. In years with small precipitation, the habitats will be greatly reduced in extent, though the amount of standing water is influenced by the dryness of the land to some degree, as indicated by Rempel (1953), and the reverse is also the case. The change of the habitats determines the area of breeding and oviposition places available for mosquitoes. Also, larval mortality has a close association with the amount of precipitation

in some circumstances, since it is often observed that pools dry up before mosquitoes emerge. Thus mosquito abundance is expected to be closely correlated to the amount of precipitation. Temperature is also an important factor influencing mosquito populations, as indicated by Rempel (1953).

From the above statement, it is clear that there exists a close relationship between mosquito abundance and meteorological factors. However, the situation will differ from species to species, as evidenced by the fact that some species appear abundantly in one year and others in another year. The analysis of these correlations over a long period will help in studies on the population dynamics.

Another approach to studies on population dynamics is the analysis of mortality factors in the field, and also the influence of various environmental conditions such as trophic factors, population density, and so on, on the fecundity of adults in the field and also in the laboratory.

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PART II. EFFECT OF LARVAL DENSITY ON THE DEVELOPMENT OF *Aedes aegypti* (L.) AND THE SIZE OF ADULTS

1. INTRODUCTION

The effect of population density on the physiology and ecology of insects has received much attention by many investigators, as it is of basic importance in the study of population dynamics. As for mosquitoes, it is known that high larval densities are associated with high larval mortality, prolongation of the larval period, and small size of resulting adults with *Aedes aegypti* (L.) (Bar-Zeev, 1957; Shannon and Putnam, 1934), *Anopheles gambiae* Giles (Gillies and Shute, 1954), and *Anopheles quadrimaculatus* Say (Terzian and Stahler, 1949). Also Spielman (1957) and Krishnamurthy and Laven (1961) reported that overcrowding larvae of *Culex pipiens* L. f. *molestus* reduces the rate of autogeny among the resulting adults, and Gillies and Shute (1954) mentioned the change in maxillary index of *Anopheles gambiae* by larval overcrowding.

Although high larval density, or overcrowding, is often accompanied by a shortage of food, it seems to be advisable to separate the effect of density itself from that of starvation, since the two could be quite different processes. Shannon and Putnam (1934) seem to have made their experiments by increasing the larval density and keeping the food amount per container constant. If so, it is very likely that the larvae in high density were affected not only by the density itself, but also by the shortage of food. Bar-Zeev (1957) used a constant amount of food per larva in his experiments to demonstrate the effect of larval density, and said, "When

the amount of food was not too high, and therefore, no film was formed, there was no undue mortality under crowded conditions; however, the development of the larvae was greatly delayed". This seems to have indicated the effect of density. However, he added "The growth rate was normal, provided that the amount of food per larva was adequate, and that the water was renewed so as to prevent the development of a film of yeast. It can, therefore, be concluded that the inhibitory effect of crowded conditions on larval development is due to lack of food".

Thus it seems that no conclusion has been established for the effect of larval density itself in mosquitoes, and therefore, it was considered worthwhile to explore this further.

The effect of density would be investigated in an experiment with a constant quantity of food per individual at varying density levels (Klomp, 1964). On the other hand, if the quantity of food per container is kept constant, the larvae at high density will suffer shortage of food particularly in the latter part of development, as well as the effect of high density. In order to recognize the effect of food quantity free from the effect of density, food quantity would have to be changed at the same density level.

2. METHOD OF EXPERIMENTS

The mosquitoes used were <u>Aedes aegypti</u> kept at the Department of Entomology, University of Alberta. The eggs, not older than 15 days from oviposition, were allowed to hatch in water with a small quantity of dried yeast (Fleishmann's). The larvae which hatched within 12 hours

were put into cups with 100 ml water containing dried yeast or rabbit pellets (North West Mill and Feed Co., Ltd.) or both. These cups were kept at constant temperatures, and the observations were made at a certain time every day. At each observation time, distilled water was added to keep a constant volume. When pupation occurred, the pupae were put into water in small glass vials with cotton plugs after recording their number, and emergence was awaited.

Four experiments were performed.

Experiment I. This was preliminary in nature. Density range was 1 to 64 larvae per cup, food used was yeast with quantity range of 1 to 64 units (1 unit = 1.7 mg) per cup, temperature, 25.7 ± 1.5 C.

Experiment II. In this experiment, the quantity of food per cup was kept constant at various density levels. Density range was 1 to 128 larvae per cup, food used was 64 units of yeast plus 100 units of rabbit pellets per cup, temperature, 29.8 ±1.2 C. From this experiment, the combined effect of food quantity and larval density will be seen.

Experiment III. This experiment was done to see the effect of different foods, that is 64 units yeast, 100 units rabbit pellets, 64 units yeast plus 100 units rabbit pellets, and 64 units yeast plus 200 units rabbit pellets. Density was kept constant at 16 larvae per cup, temperature, 29.8 ±1.2 C.

Experiment IV. In this experiment, the quantity of yeast per larva was kept at 1 and 4 units, density range 1 to 256, temperature, 26.3 ± 0.9 C. Thus the effect of larval density will be seen from the data

based on series of density levels at constant food quantity per larva. Also, by comparing in the same density level, the effect of food quantity will be demonstrated.

3. RESULTS OBTAINED

3.1. Effect of larval density on larval and pupal mortalities

The larval and pupal mortalities in Experiments I, II, III, and IV are given in Tables 1, 2, 3, and 4, respectively.

In experiment I, low larval mortality was observed at the density levels of 1 and 4 larvae per cup, when 4 to 64 units of yeast were supplied to each cup. With increasing density particularly when the amount of yeast was small, larval mortality became higher. No pupation occurred in the density 16 with 4 units of yeast per cup or in the density 64 with 4 or 16 units. No appreciable tendency was recognized in pupal mortality.

Experiment II gave generally high pupation rate throughout the density levels of 1 to 128, indicating that the food used, 64 units yeast plus 100 units rabbit pellets, is suitable for larval survival. However, the larval mortality is lower at density 16 than at other densities, and this seems to indicate the optimum density for larval survival, with this combination of quantity and quality of the food.

Experiment III, where the density of larvae was 16 per cup, shows that larval and pupal mortalities decrease from 64 units yeast to 64 units yeast plus 200 units rabbit pellets. This means that the lower food shown in the table is the better food for larval and pupal survival.

Table 1. Mortalities of <u>Aedes aegypti</u> larvae and pupae reared at different densities with different amounts of yeast (Experiment I).

Density	Yeast (units)* per cup	Yeast (units)* per larva	Replicate	Total No. of larvae	Larval mortality (%)	No. of pupae Male	No. of pupae Female	No. of pupae Total	Pupal mortality (%)
1	4	4	6	6	0.0	3	3	6	16.7
1	16	16	6	6	0.0	4	2	6	0.0
1	64	64	6	6	0.0	3	3	6	0.0
4	4	1	4	16	18.7	8	5	13	0.0
4	16	4	4	16	18.7	6	7	13	15.4
4	64	16	4	16	25.0	9	3	12	0.0
16	4	1/4	1	16	100.0	0	0	0	-
16	16	1	1	16	43.7	4	5	9	22.2
16	64	4	1	16	12.5	6	9	15	6.7
64	4	1/16	1	64	100.0	0	0	0	-
64	16	1/4	1	64	100.0	0	0	0	-
64	64	1	1	64	54.7	20	9	29	3.4

* 1 unit = 1.7 mg

Table 2. Mortalities of _Aedes aegypti_ larvae and pupae reared at different densities with a constant amount of food per cup (Experiment II).

Density	Replicate	Total No. of larvae	Larval mortality (%)	No. of pupae Male	Female	Total	Pupal mortality (%)
1	23	23	13.0	12	8	20	5.0
4	12	48	6.2	21	24	45	4.4
16	5	80	1.2	43	36	79	1.3
64	3	192	3.6	100	85	185	1.6
128	2	256	16.4	126	88	214	0.9

Food used: 64 unit yeast plus 100 unit rabbit pellets per cup (1 unit = 1.7 mg).

Table 3. Mortalities of <u>Aedes</u> <u>aegypti</u> larvae and pupae reared with different foods (Experiment III).

Food used*	Replicate	Total No. of larvae	mortality (%)	No. of pupae Male	Female	Total	Pupal mortality (%)
Y64	2	32	12.5	12	16	28	7.1
R100	2	32	9.4	15	14	29	3.4
Y64 + R100**	5	80	1.2	43	36	79	1.3
Y64 + R200	2	32	0.0	19	13	32	0.0

Density: 16 larvae per cup.

*Y: yeast; R: rabbit pellets; accompanied figure: quantity per cup in units (1 unit = 1.7 mg).

** Data are from Table 2.

Table 4. Mortalities of Aedes aegypti larvae and pupae reared at different densities with 2 series of a constant amount of yeast per larva (Experiment IV).

Density	Yeast (units)* per cup	Yeast (units)* per larva	Replicate	Total No. of larvae	Larval mortality (%)	No. of pupae: Male	No. of pupae: Female	No. of pupae: Total	Pupal mortality (%)
1	1	1	32	32	9.4	18	11	29	3.4
4	4	1	13	52	20.8	23	19	42	7.1
16	16	1	5	80	13.7	44	25	69	1.4
64	64	1	2	128	57.8	54	0	54	1.9
256	256	1	2	512	86.9	64	3	67	40.3
1	4	4	32	32	12.5	20	8	28	3.6
4	16	4	13	52	0.0	27	25	52	0.0
16	64	4	5	80	13.7	42	27	69	2.9
64	256	4	2	128	23.4	60	38	98	5.1
256	1024	4	2	512	97.1	8	7	15	0.0

* 1 unit = 1.7 mg

In Experiment IV, two series of the amount of yeast, that is 1 and 4 units per larva, were used. When the density was 16 or less, fairly high pupation was obtained, though the mortality is slightly higher with 1 unit yeast per larva than with 4 units. In density 64 with 1 unit yeast per larva, that is 64 units per cup, larval mortality was more than 50%, and only males pupated. In the density of 256 with 1 unit yeast per larva, that is 256 units per cup, larval mortality further increased up to 87%, 40% of pupae failed to emerge, and very low proportion of females was obtained. Very high larval mortality was observed also in the density of 256 with 4 units of yeast per larva, that is 1024 units per cup. This amount of yeast seemed to be too much for 100 ml water, because a film was formed on the water surface and high mortality occurred in earlier instars, unlike other combinations of density and food amount. Thus such a very low pupation rate as 2.9% is not due to the effect of high larval density, but probably to the film formation or other unfavorable conditions of the culture medium.

In short, larval mortality generally increases with increased density and decreased food quantity through the shortage of food and the larval density itself. There seems to be an optimum density for larval survival, which differs from the minimum density. If the conditions are not suitable, then the favored sex is the male.

3.2. Effect of larval density on pupation curve

Frequency curves of pupation by sex in four experiments are shown in Figs. 1, 2, 3, 4, and 5. Males pupated earlier than females

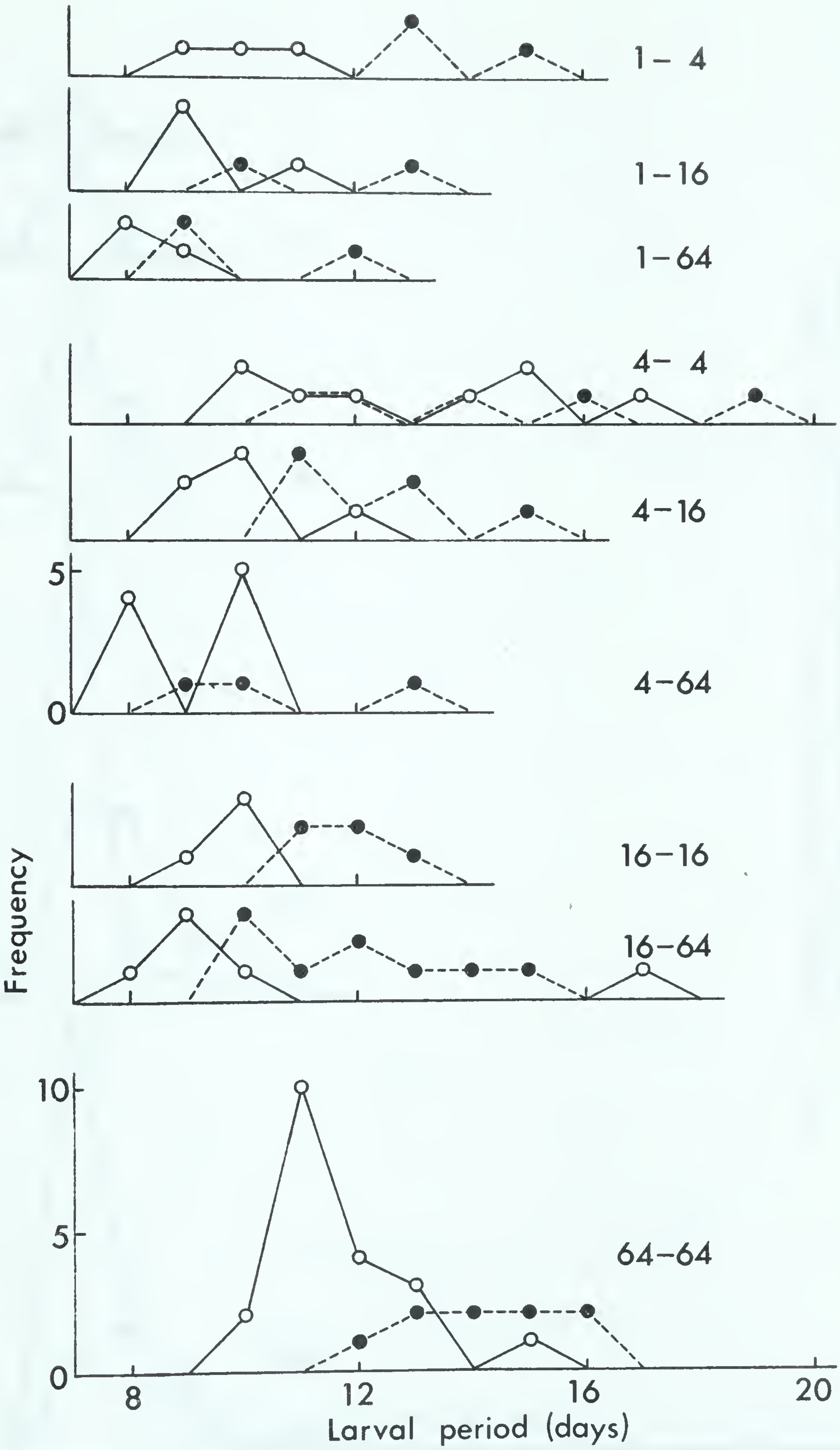


Fig. 1. Frequency distributions of larval period of *Aedes aegypti* (Experiment I). 4-64, for example, indicates that the larval density is 4 and the amount of yeast is 64 units per cup. ○ : males; ● : females.

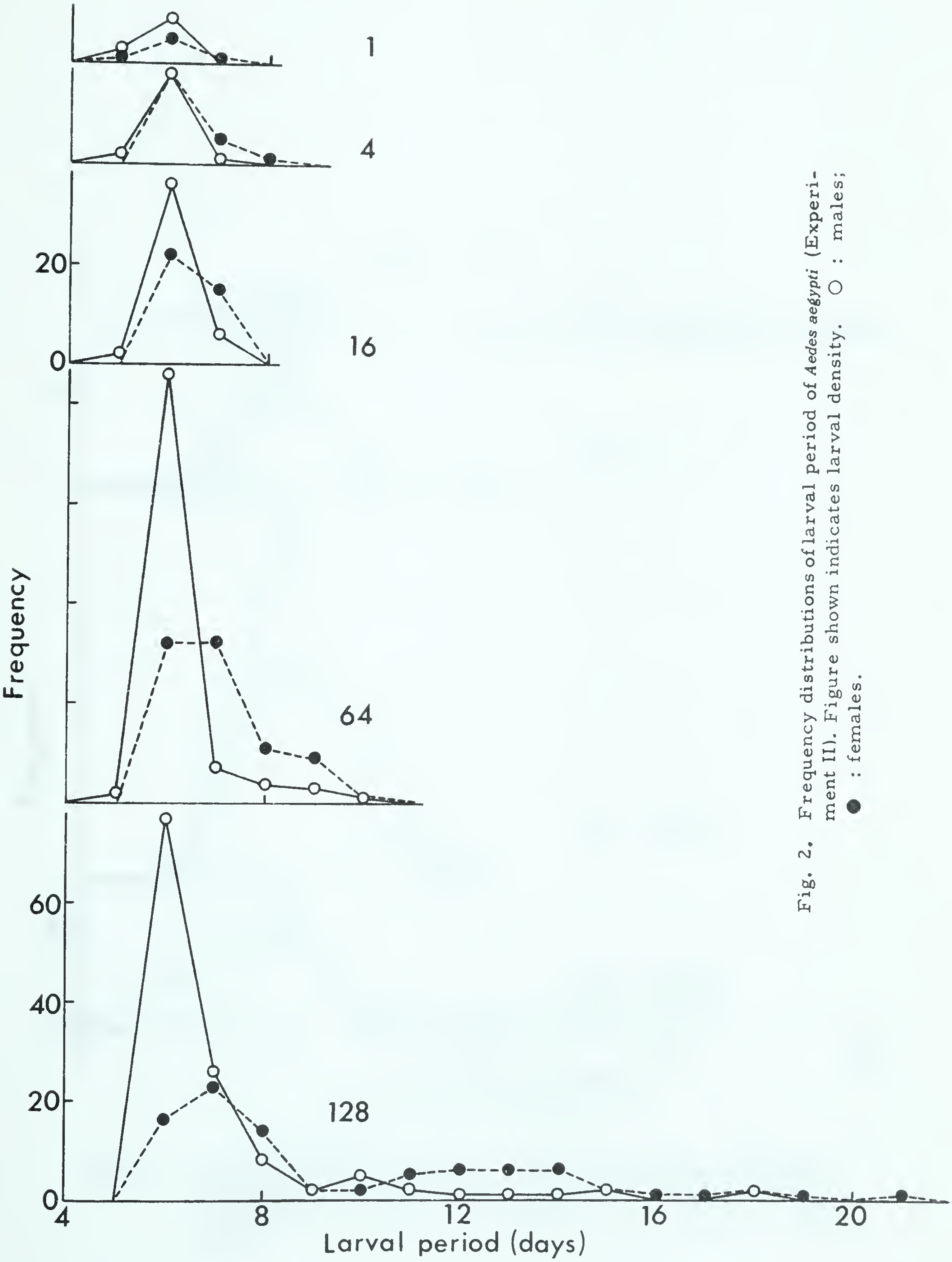


Fig. 2. Frequency distributions of larval period of *Aedes aegypti* (Experiment II). Figure shown indicates larval density. ○ : males; ● : females.

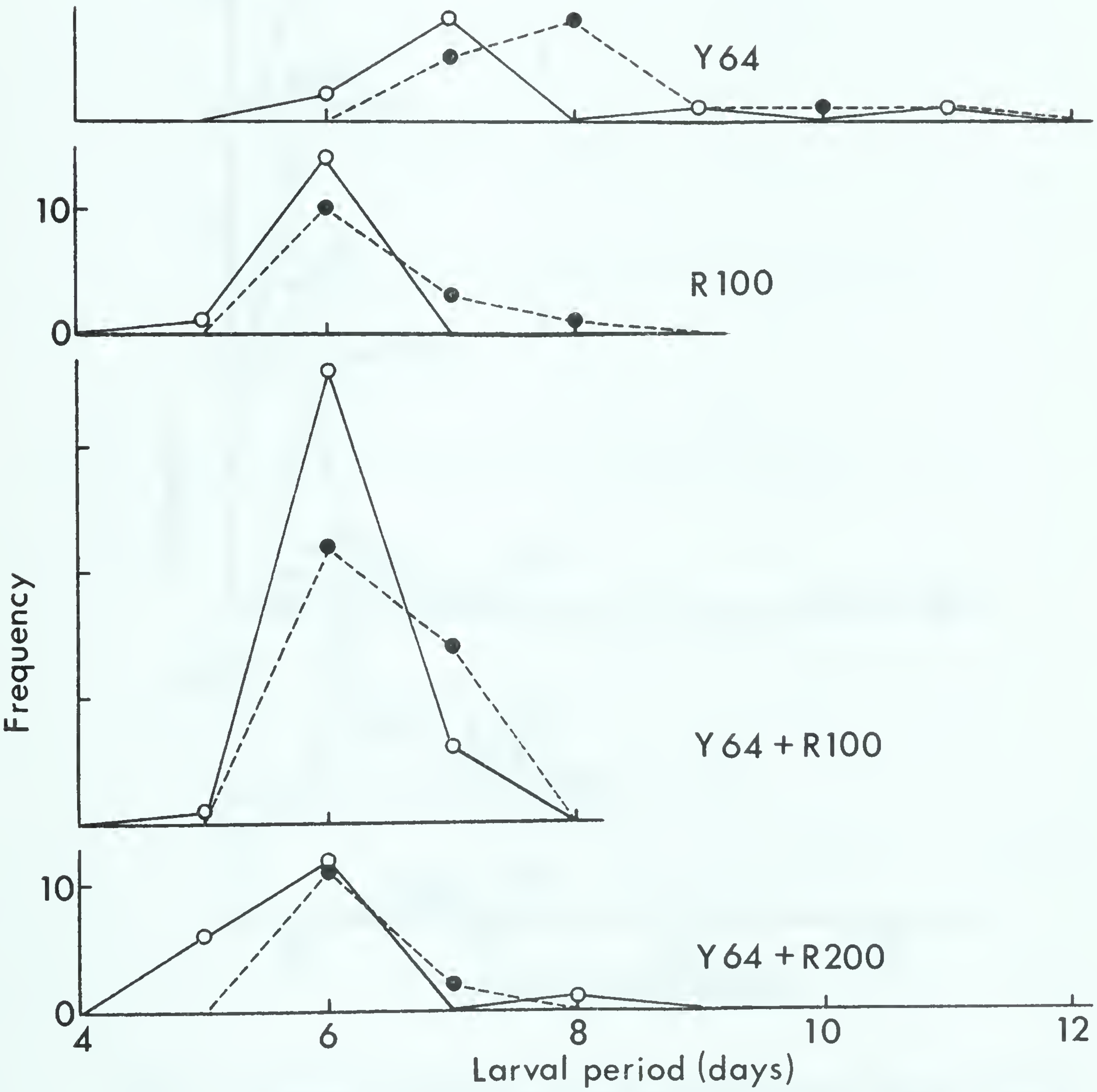


Fig. 3. Frequency distributions of larval period of *Aedes aegypti* (Experiment III). Y and R and accompanied figure indicate yeast and rabbit pellets and their amount in units. ○ : males, ● : females.

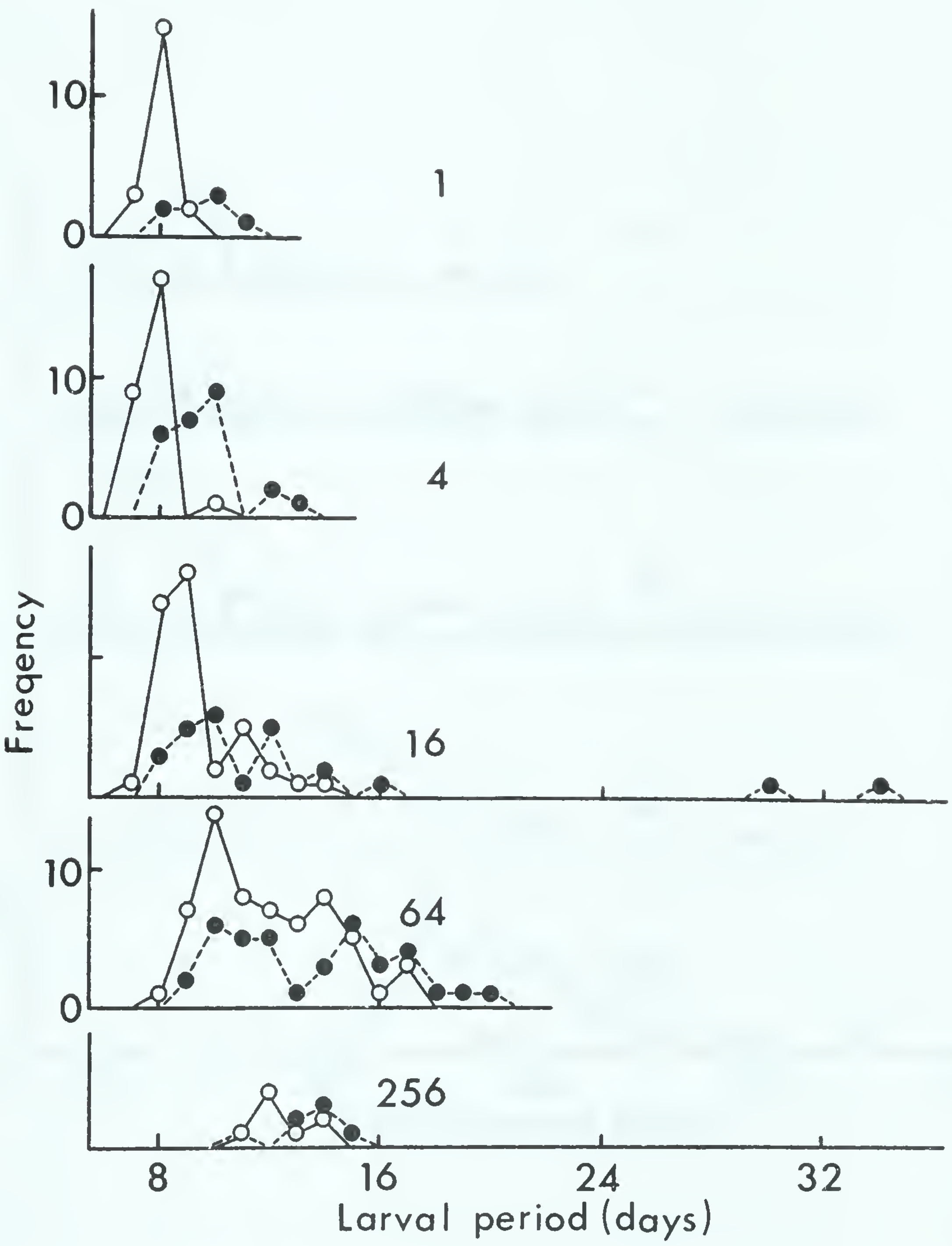


Fig. 4. Frequency distributions of larval period of *Aedes aegypti* (Amount of yeast per larva: 4 units; Experiment IV). Figure shown indicates larval density. ○ : males; ● : females.

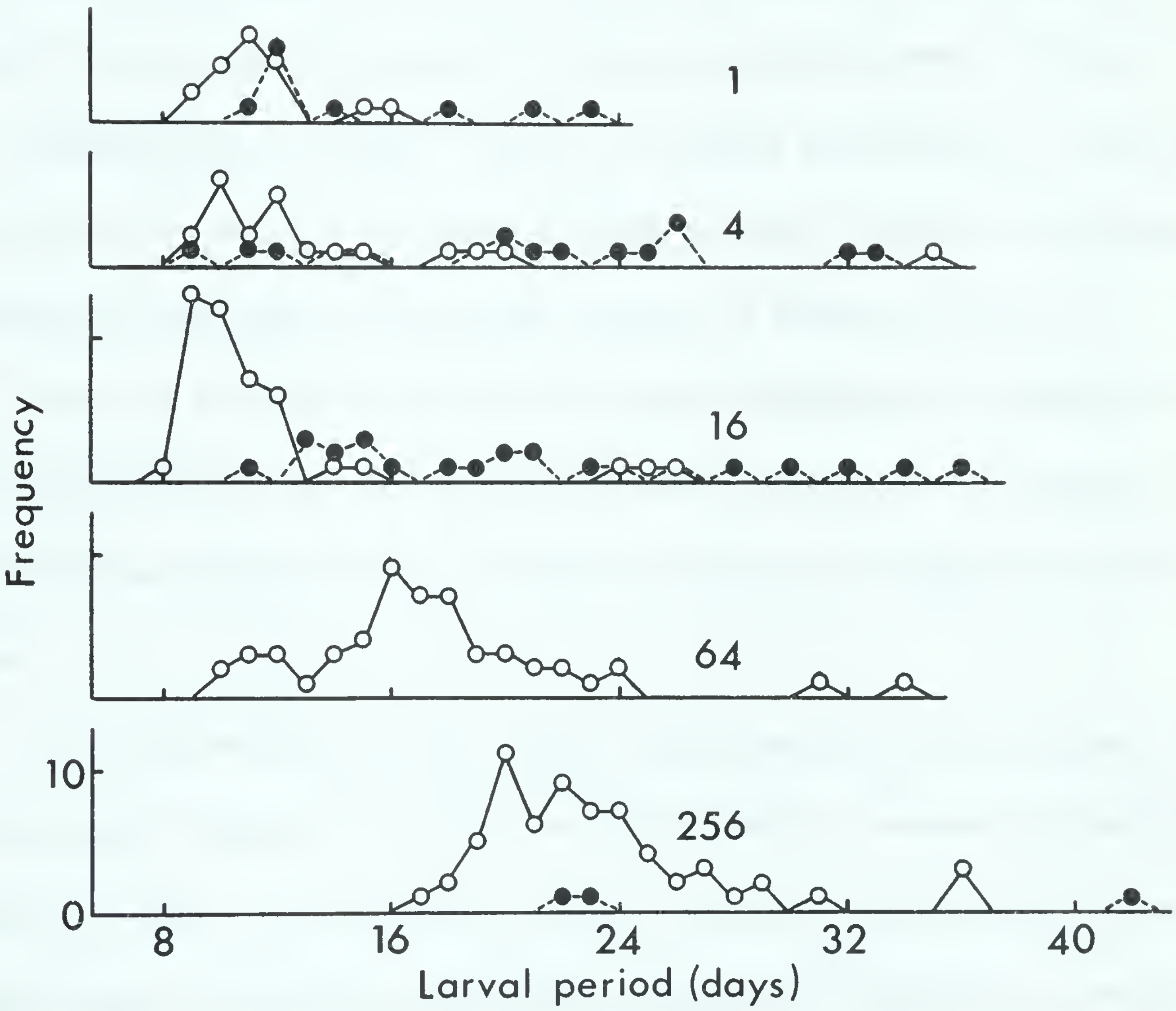


Fig. 5. Frequency distributions of larval period of *Aedes aegypti* (Amount of yeast per larva: 1 unit; Experiment IV). Figure shown indicates larval density. ○ : males; ● : females.

throughout the experiments. Generally a shorter larval period is seen in the cups where the density is lower and the amount of yeast is larger. When larval periods are compared on the basis of the same density with different amounts of food (see Fig. 1; compare Figs. 4 and 5), a longer larval period is seen with the decreased amount of food.

When the amount of food per larva was kept constant and the density of larvae was increased, the delay in development is clear, as seen in Experiment IV (Figs. 4 and 5). This is attributable to the effect of high larval density, not to the shortage of food, because the comparisons were made on the basis of the same amount of food per larva.

Here, it is apparent that the larval development is affected not only by the quantity of food, but also directly by the larval density, and the effect is more remarkable, when the amount of food per larva is smaller.

It is interesting that the longer larval period is usually associated with increased variation in larval period and with a tendency to be skewed towards the right. If a pupation curve is normally distributed, then it is expected that a cumulative percentage frequency of pupation in probit will be linear. Now, the normality of the pupation curves in Experiments II and IV, in which a fairly large number of larvae were used, was examined.

Cumulative percentage pupation in probit is plotted against larval period (days) in Figs. 6 to 11. When the density is low and food amount is large a linear relation is seen, that is, those pupation curves are shown

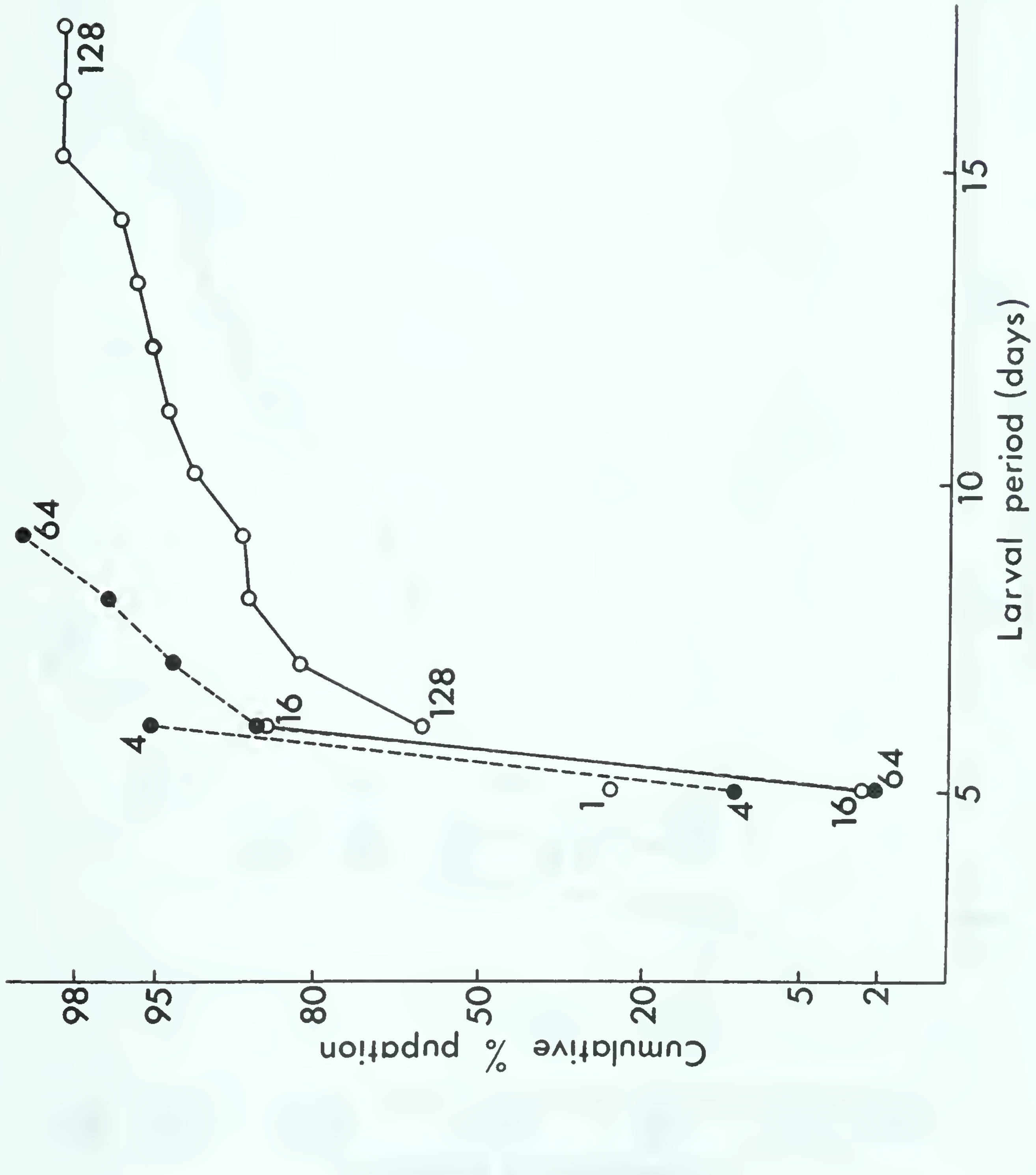


Fig. 6. The relation between cumulative percentage pupation (probit scale) and larval period in males of *Aedes aegypti* (Experiment II). Figure shown indicates larval density.

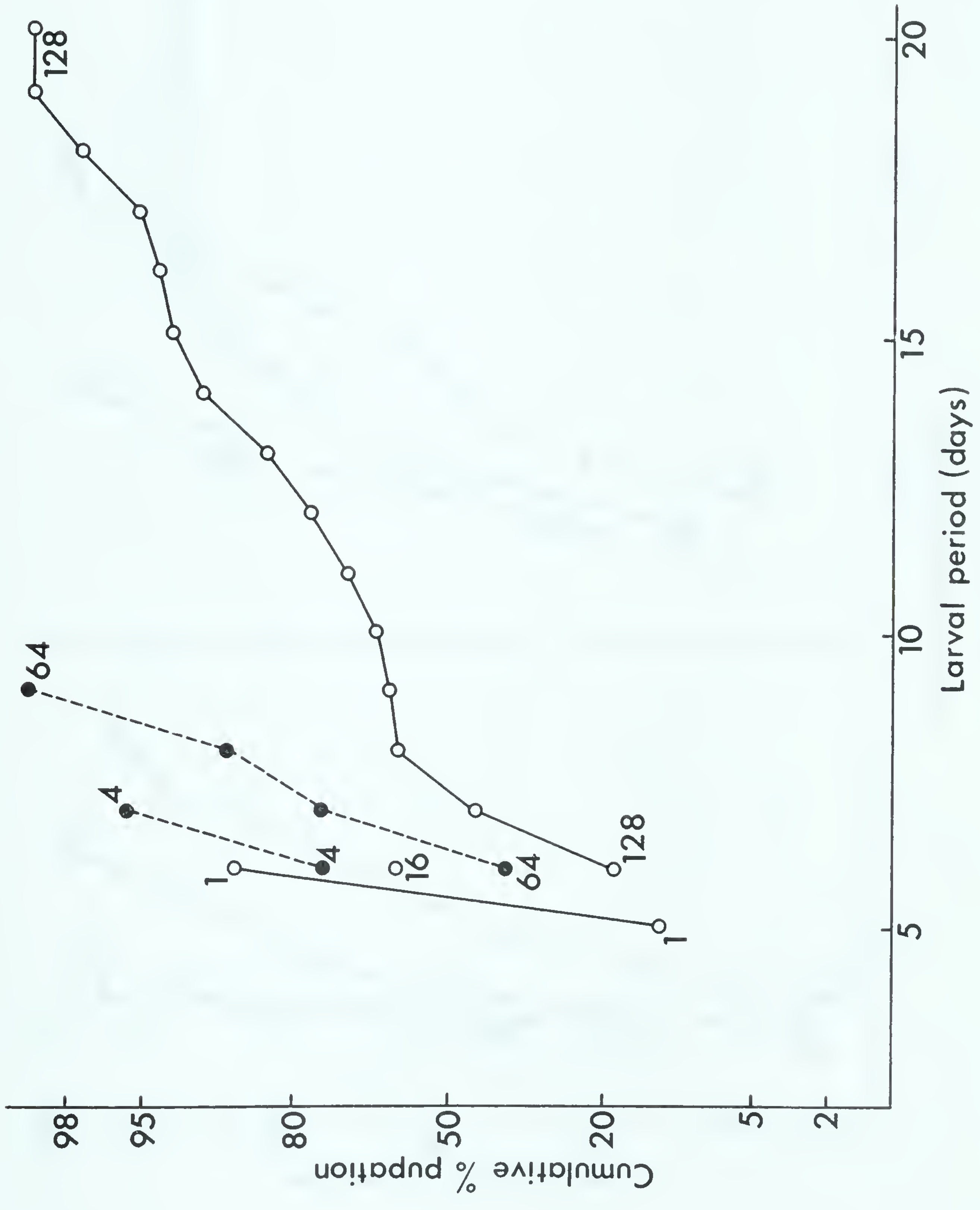
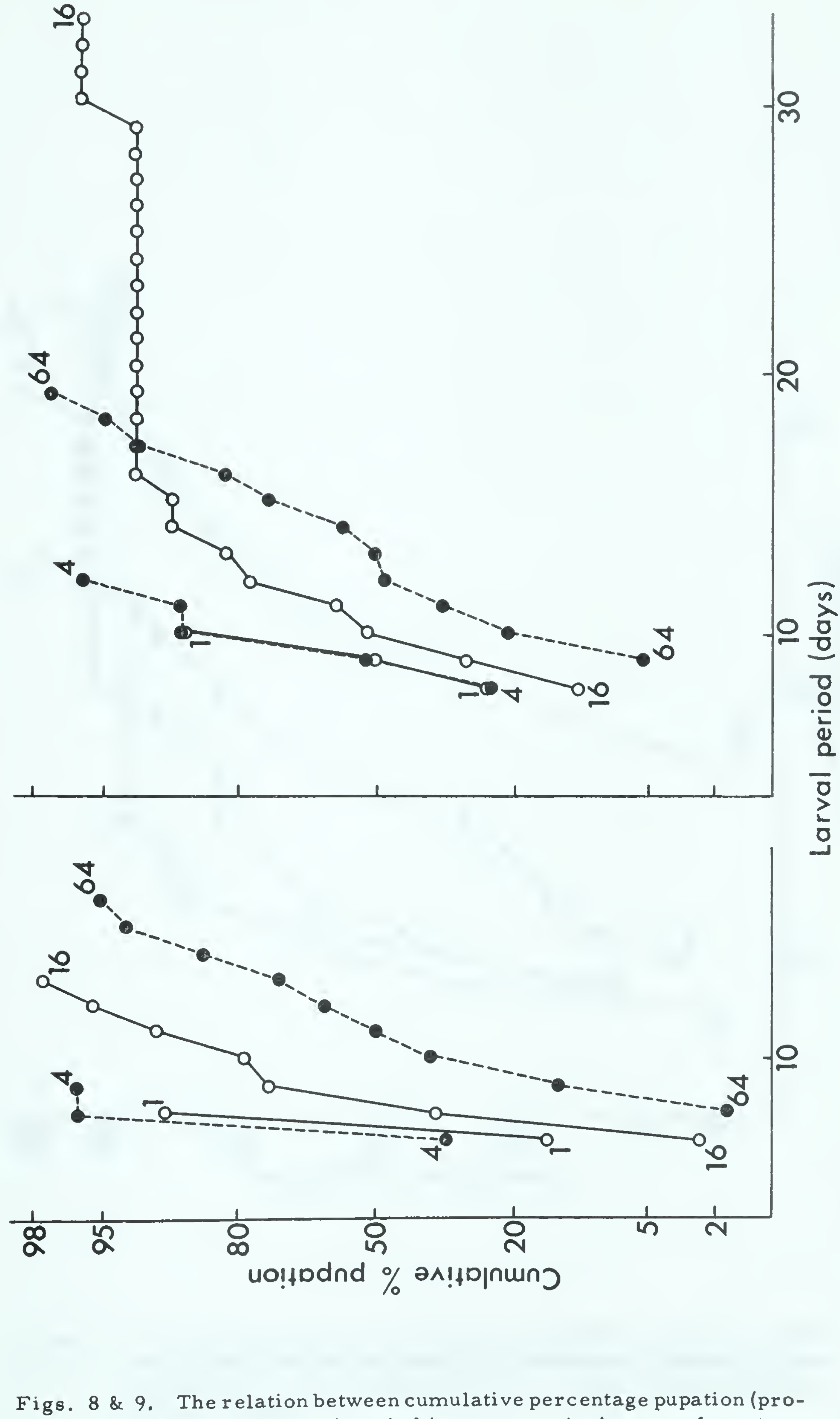


Fig. 7. The relation between cumulative percentage pupation (probit scale) and larval period in females of *Aedes aegypti* (Experiment II). Figure shown indicates larval density.



Figs. 8 & 9. The relation between cumulative percentage pupation (probit scale) and larval period in *Aedes aegypti*. Amount of yeast per larva: 4 units; Experiment IV. 8. Males, 9. Females.

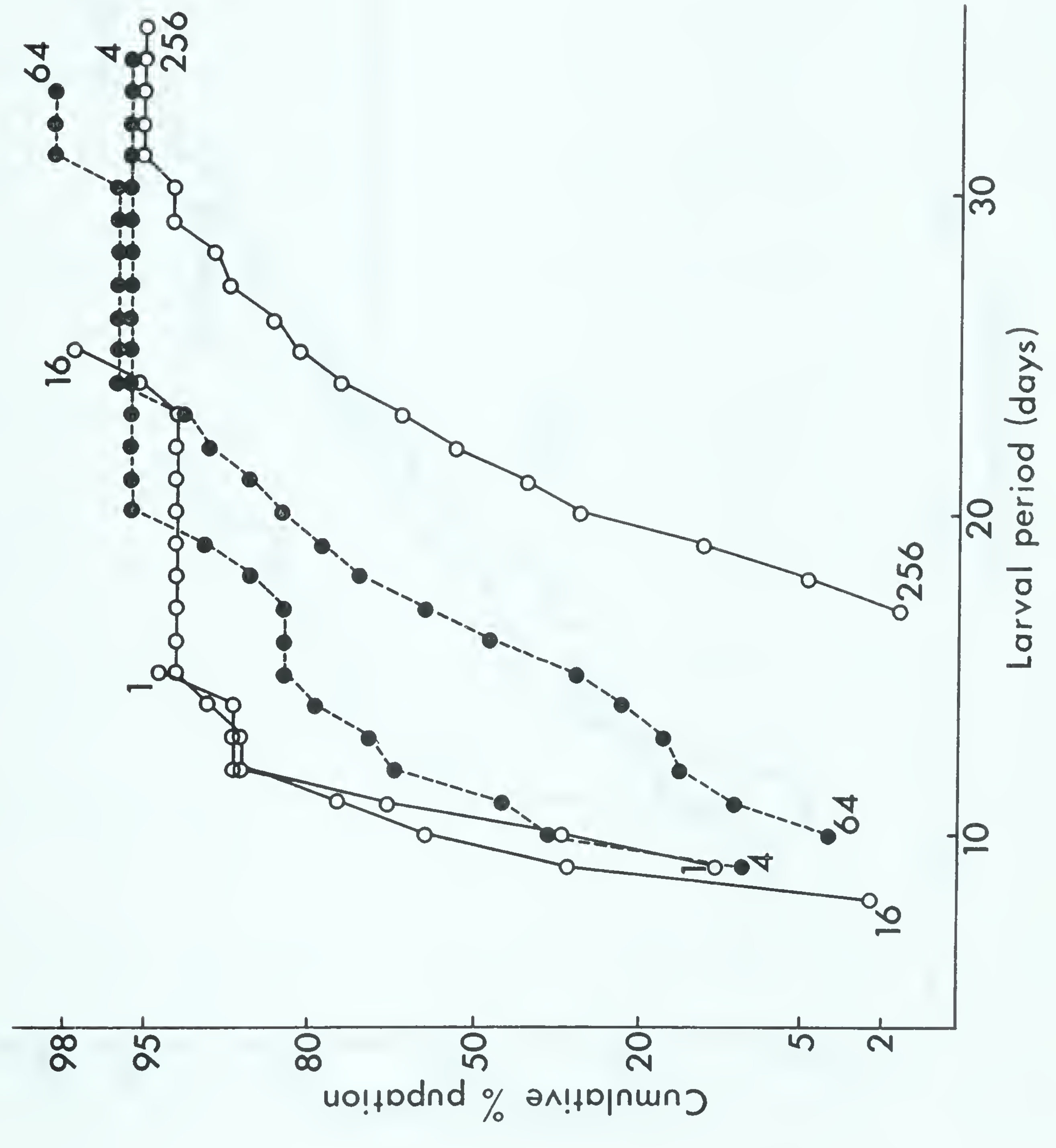


Fig. 10. The relation between cumulative percentage pupation (probit scale) and larval period in males of *Aedes aegypti* (Amount of yeast per larva: 1 unit; Experiment IV). Figure shown indicates larval density.

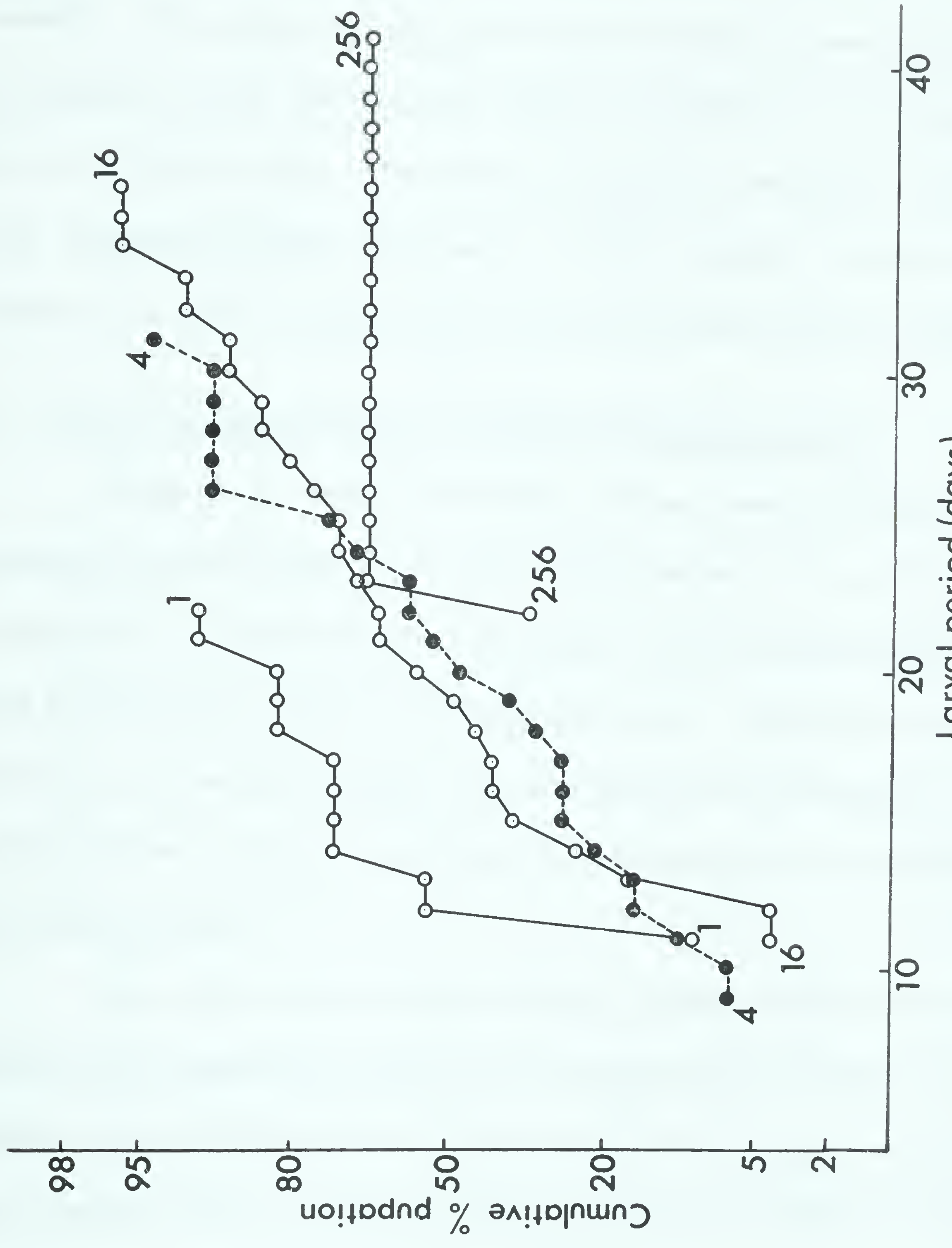


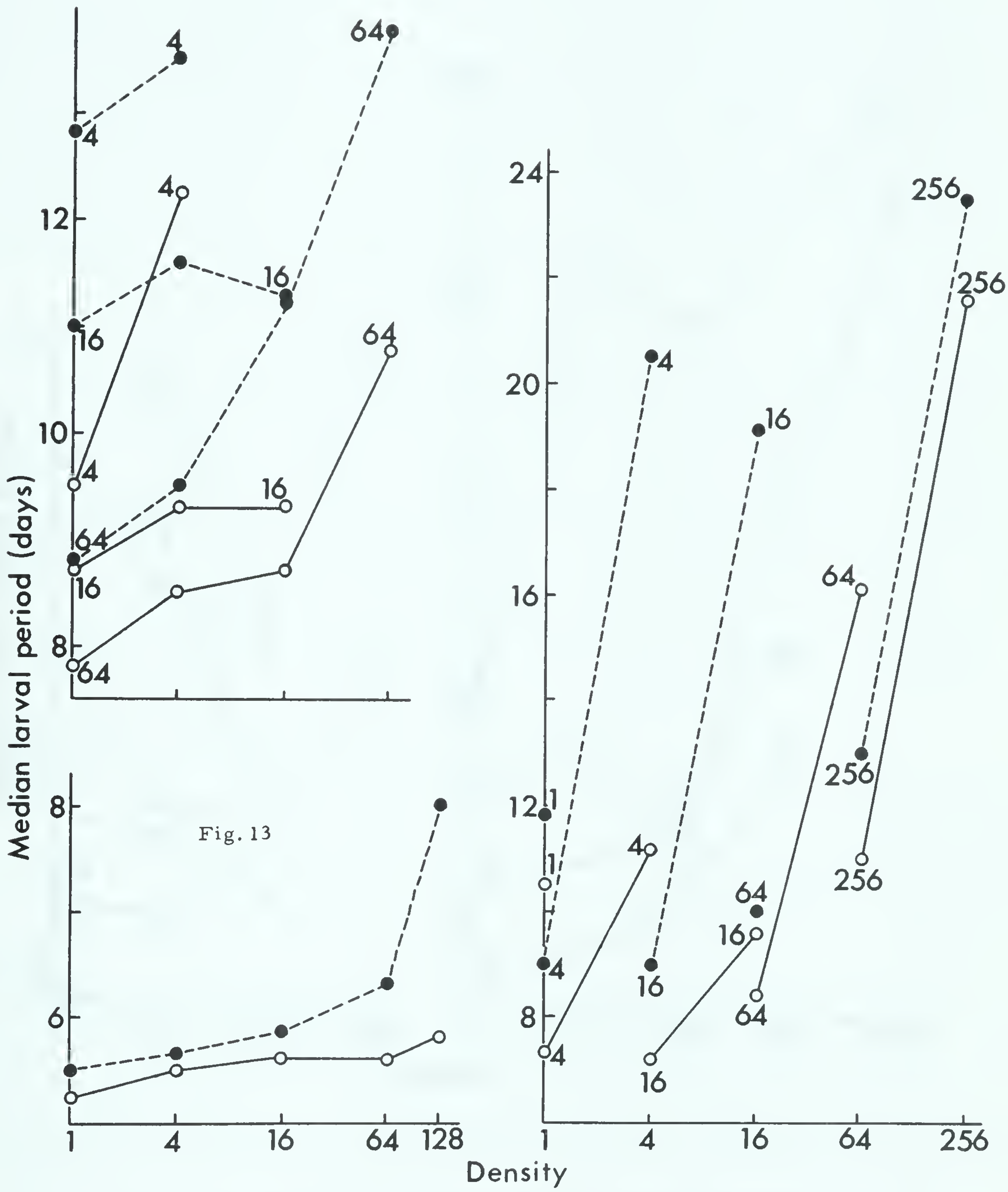
Fig. 11. The relation between cumulative percentage pupation (probit scale) and larval period in females of *Aedes aegypti* (Amount of yeast per larva: 1 unit; Experiment IV). Figure shown indicates larval density.

to follow the normal distribution. The deviation from the normal distribution becomes remarkable with increasing density and decreasing food quantity. Thus there is some deviation from the normal distribution in the pupation curve, particularly when the conditions are unfavorable for larval development. Even when conditions are good, a few individuals sometimes pupate very late. For this reason, it seems that the median is a better representative of larval period than the mean.

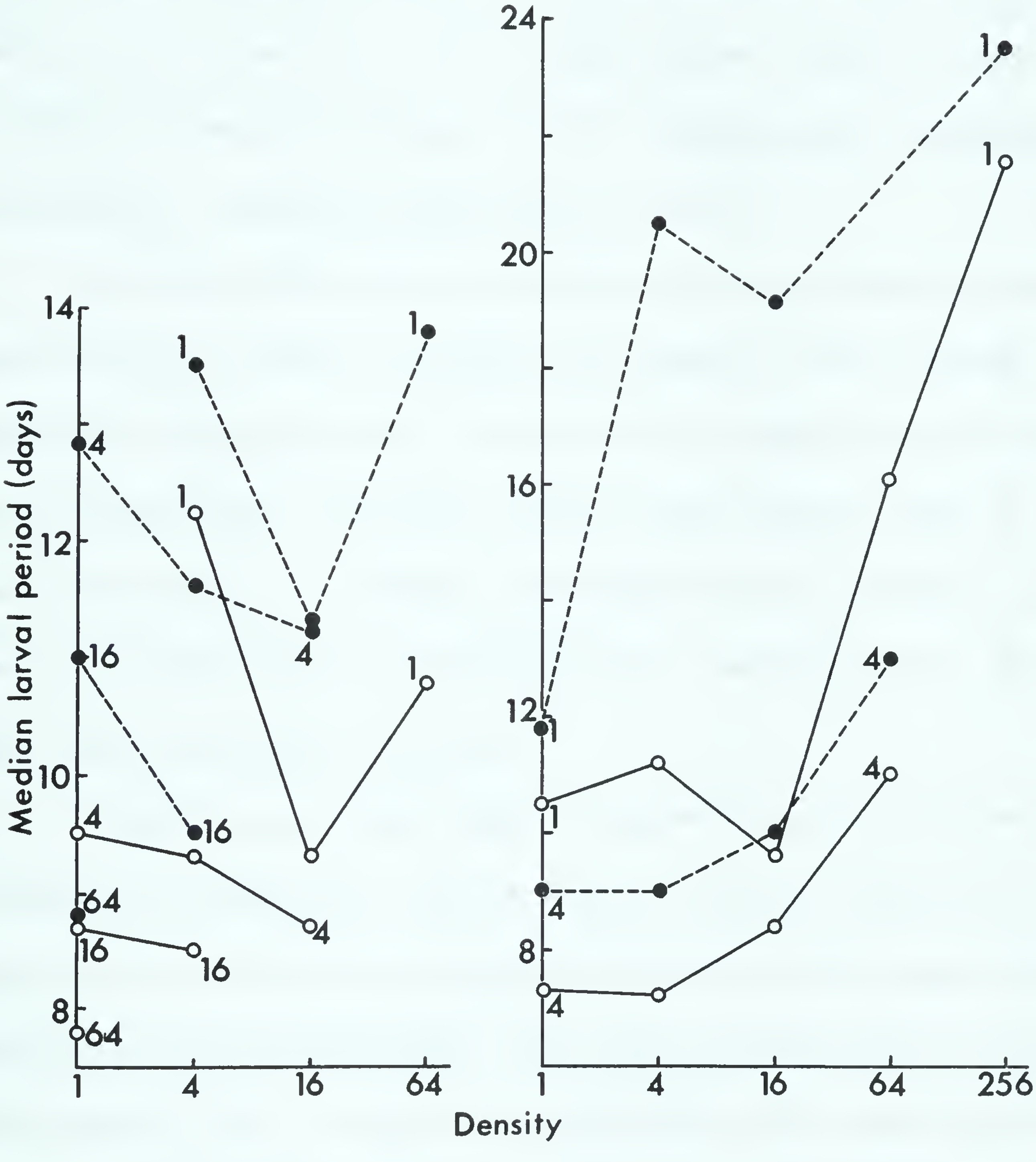
3.3. Effect of larval density on larval and pupal periods

Figs. 12, 13, and 14 show the relation between median larval period and larval density per cup for Experiment I, II, and IV, respectively. In these figures, the points with the same amount of food per cup were connected by straight lines. Generally, the median larval period becomes longer with increasing larval density. This is rather natural, because the amount of food per larva decreases with increasing density.

By connecting the points with the same amount of food per larva, the data for Experiments I and IV are represented in Figs. 15 and 16. In density levels of 256 and 64 of Experiment IV, a longer median period was obtained than in 1, 4 or 16, in spite of the fact that the amount of food available for each larva in higher densities is the same as, or even slightly larger than, in lower densities. Here, the effect of high larval density is again suggested. Also in Experiment I, the tendency of the median to increase is seen at the density levels of 64 or more. It is interesting that there seems to exist a valley in median larval period at



Figs. 12-14. Median larval period of *Aedes aegypti* at each density level. Points for the same amount of yeast per cup are connected by lines. 12. Experiment I. 13. Experiment II. 14. Experiment IV. Figure shown indicates the units of yeast per cup.
○ : males; ● : females.



Figs. 15 & 16. Median larval period of *Aedes aegypti* at each density level. Points for the same amount of yeast per larva are connected by lines. 15. Experiment I. 16. Experiment IV. ○ : males; ● : females.

density 16, particularly when the amount of food is small, and furthermore, in the food amount of 1 in Experiment IV, the median becomes again smaller at density 1 than 4. The reasons for such peculiarities of the curves are not clear, but it seems that the median is determined by a balance between the effects of larval density and the amount of food available, and perhaps some other factors.

No distinct difference in pupal period was recognized among various amounts of food nor among larval density levels, though pupal density may affect the period. It seems that the pupal period is affected only by temperature, or at least, if some other factors affect it, their effect is very small. In Table 5, mean pupal periods in days are given by sex at the three different temperatures. The female has a slightly longer pupal period than the male.

It would be practically right to suppose that the larval period is determined by temperature, larval density, and the conditions of culture medium such as the quality and quantity of food, but the pupal period is determined only by temperature. The ratio of larval period to pupal period seems, then, to indicate the suitability of the conditions for larval development. This ratio may be used to compare the larval period, even when experiments were made at different temperatures.

The calculated values for the ratio are shown in Tables 6 and 7, and compared on the basis of the same combinations of larval density and food amount in different experiments. The ratios for the combinations of D1Y4 (density 1 larva per cup, yeast 4 units per cup), D4Y16,

Table 5. Pupal periods of Aedes aegypti by sex at different temperatures.

Experiment	Temperature C	Mean pupal period (days)	
		Male	Female
I	25.7	2.76	2.78
II and III	29.8	1.83	1.94
IV	26.3	2.36	2.42

Table 6. The ratio of larval to pupal period of the males of *Aedes aegypti* (Experiments I - IV).

Density	Food used*	Exp. I	Exp. II	Exp. III	Exp. IV	Mean
1	Y1				4.45	4.45
	Y4	3.44			3.14	3.28
	Y16	3.15				3.15
	Y64	2.83				2.83
	Y64 + R100		2.92			2.92
4	Y4	4.53			4.79	4.66
	Y16	3.37			3.05	3.21
	Y64	3.08				3.08
	Y64 + R100		3.01			3.01
16	Y4	**				**
	Y16	3.37			4.07	3.72
	Y64	3.15		3.55	3.56	3.42
	R100			2.98		2.98
	Y64 + R100		3.07			3.07
	Y64 + R200			2.89		2.89
64	Y4	**				**
	Y16	**				**
	Y64	3.91			6.86	5.39
	Y256				4.66	4.66
	Y64 + R100		3.07			3.07
128	Y64 + R100		3.19			3.19
256	Y256				9.24	9.24
	Y1024				5.00***	5.00***

* See Table 3.
** Unable to pupate.
*** Film was formed on water surface, larval mortality was very high.

Table 7. The ratio of larval to pupal period of the females of *Aedes aegypti* (Experiments I - IV).

Density	Food used*	Exp. I	Exp. II	Exp. III	Exp. IV	Mean
1	Y1				4.88	4.88
	Y4	4.60			3.72	4.16
	Y16	3.96				3.96
	Y64	3.17				3.17
	Y64 + R100		2.84			2.84
4	Y4	4.86			8.47	6.62
	Y16	4.17			3.72	3.95
	Y64	3.42				3.42
	Y64 + R100		2.91			2.91
16	Y4	**				**
	Y16	4.06			7.93	6.00
	Y64	4.06		3.80	4.09	3.98
	R100			2.94		2.94
	Y64 + R100		3.02			3.02
	Y64 + R200			2.88		2.88
64	Y4	**				**
	Y16	**				**
	Y64	4.96			**	>4.96
	Y256				5.37	5.37
	Y64 + R100		3.25			3.25
128	Y64 + R100		4.12			4.12
256	Y256				9.75****	9.75****
	Y1024				5.45***	5.45***

* See Table 3.
, * See Table 6.
**** Only three females pupated.

and D16Y64 agree quite well among experiments, but those for D4Y4, D16Y16, D64Y16, and D64Y64, are rather different from one another. The number of larvae used in Experiment I was not sufficient, and the latter combinations are considered somewhat unsuitable so that very slight differences in the conditions will make rather great changes in larval development. These would be responsible for rather great difference of the ratios in the latter group of combinations.

The above procedure will be valid only if the ratio of larval period to pupal period is constant over a reasonable temperature range. For this reason, further studies are required to determine the usefulness of the ratio. However, it is clear from the tables that larval period varies greatly with the quantity and quality of food at the same density level, and also that the same amount of food per cup, or even per larva, does not give the same larval period at different density levels. Therefore, care should be taken in attempting to determine the larval period at a certain temperature, or the developmental zero of mosquito larvae by rearing them at different temperatures.

3.4. Effect of larval density on body size of resulting adults

In Figs. 17 and 18, the frequency distributions of wing length of the resulting adults in Experiments II and IV are given.

In Experiment II (Fig. 17), the wing length increases in both sexes slightly from density 1 to 16 larvae per cup, and decreases greatly with increasing density from 16. Fig. 18 shows the similar situation in Experiment IV, except for density 256 with yeast 4 units per larva, where

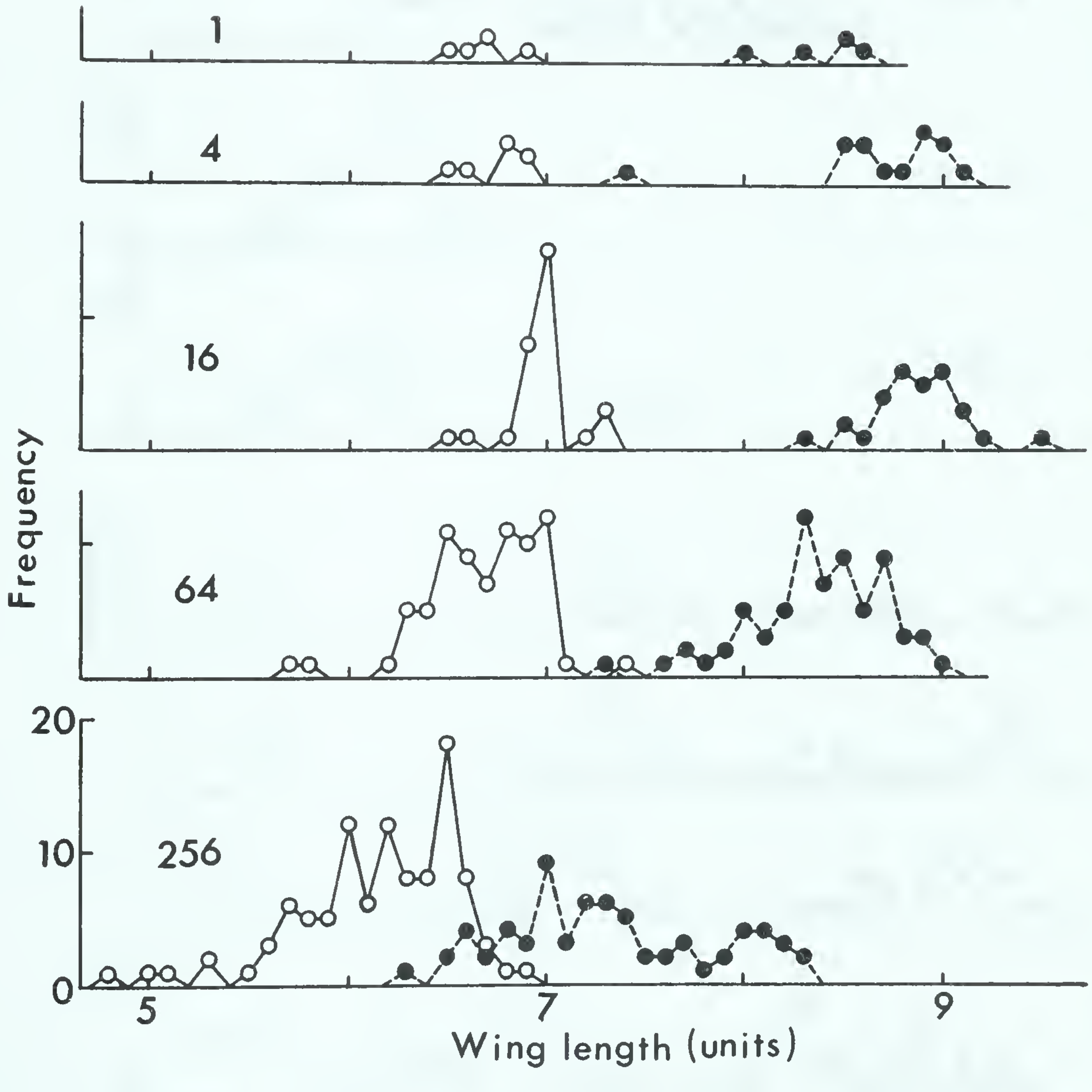


Fig. 17. Frequency distributions of wing length of *Aedes aegypti* (Experiment II). Figure indicates larval density. ○ : males; ● : females.

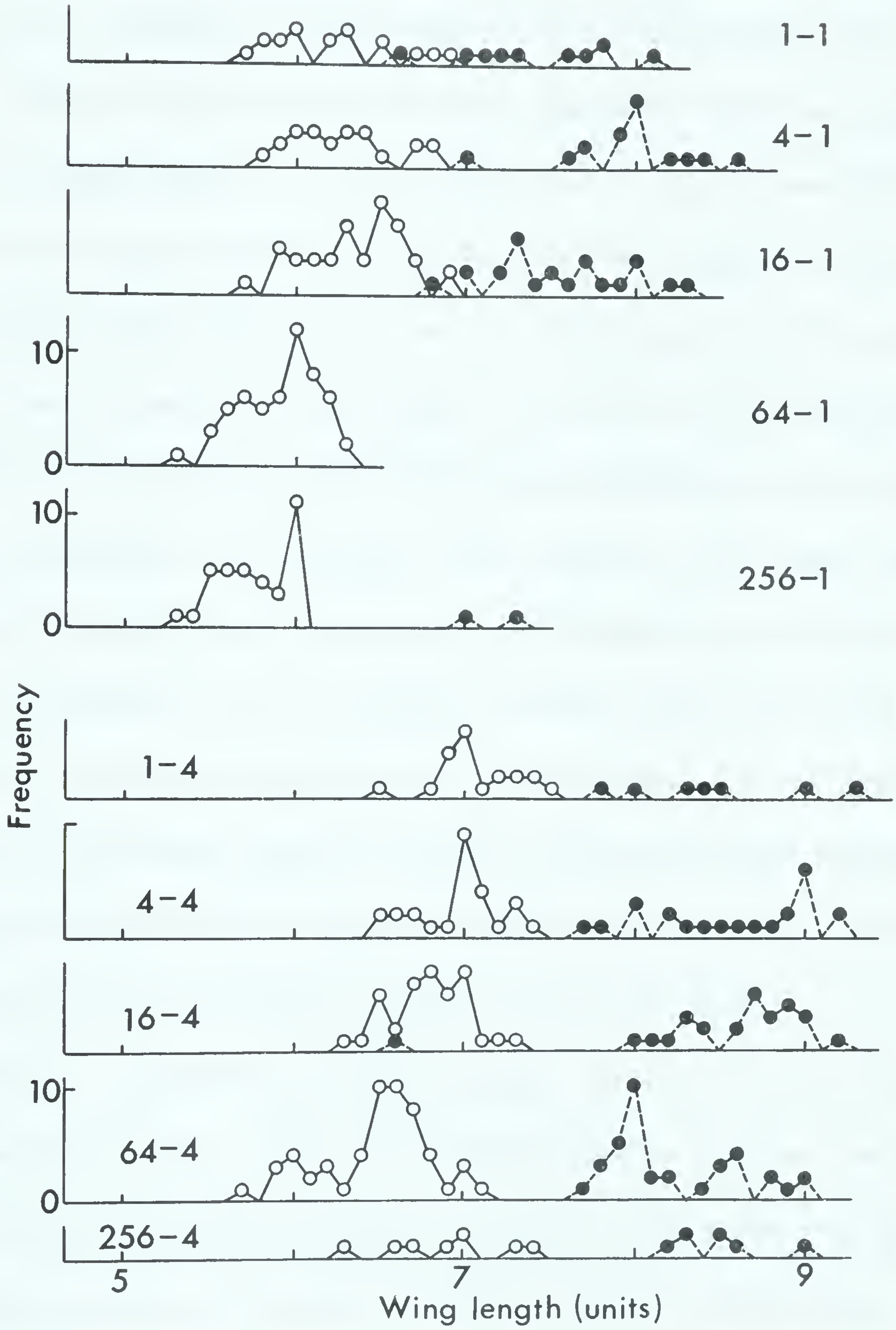


Fig. 18. Frequency distributions of wing length of *Aedes aegypti* (Experiment IV). 16-4, for example, indicates that the larval density is 16 and the amount of yeast is 4 units per larva. ○ : males; ● : females.

the wing length is not considered to reflect the effect of this density, owing to high larval mortality in the earlier instars, as mentioned earlier. However, the changes in wing length are less remarkable than in Experiment II. This is due to the fact that the quantity of food per cup was kept constant in Experiment II, on the other hand in Experiment IV the quantity per larva was kept constant. Nevertheless, the apparent effect of larval density on the wing length can be seen in Experiment IV (Fig. 18).

It seems that the wing length of females is more sensitively affected than that of males with decreasing suitability for the larval stage, so that considerable overlapping in wing length of both sexes appears, as for example between densities 64 and 128 in Experiment II (Fig. 17). When the conditions become still less suitable, only males will pupate, as indicated from the densities 64 and 256 with yeast 1 unit per larva.

It is interesting that the frequency curve becomes steeper at the right hand side with decreasing suitability in the conditions for larval development, but the reasons for this are not yet clear.

Figs. 19 and 20 show the frequency curves of thorax length in Experiments II and IV. The thorax length shows a similar tendency to the wing length, excepting that the steepness of the curves at the right hand side is not seen, when the conditions become unfavorable.

In Fig. 21, the relation between mean wing length and mean thorax length is shown for each density level in Experiment II and for each type of food in Experiment III. In density level of 16 or more in Experiment II, both wing and thorax decrease in length along a straight line with

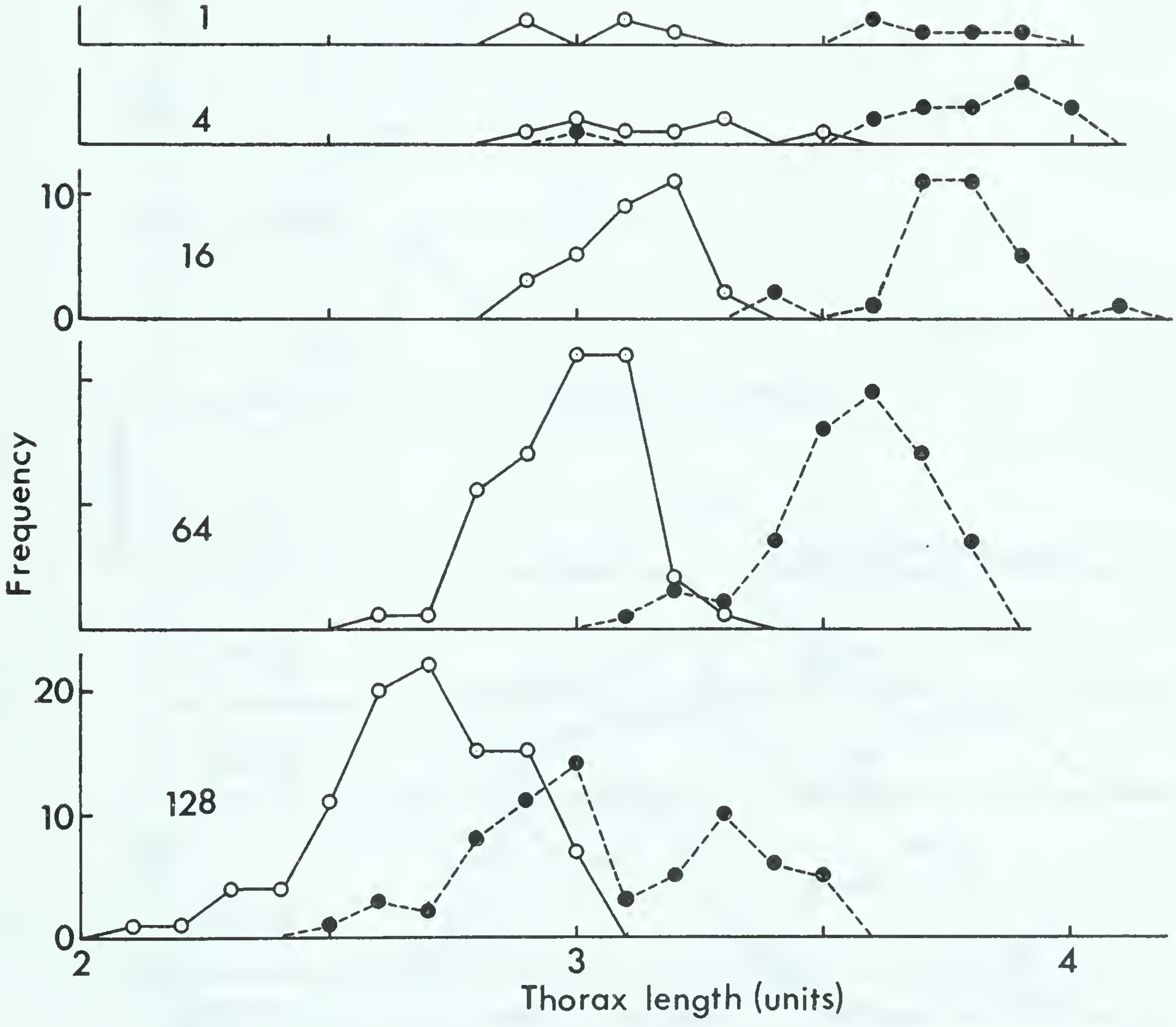


Fig. 19. Frequency distributions of thorax length of *Aedes aegypti* (Experiment II). Figure shown indicates larval density. ○ : males; ● : females.

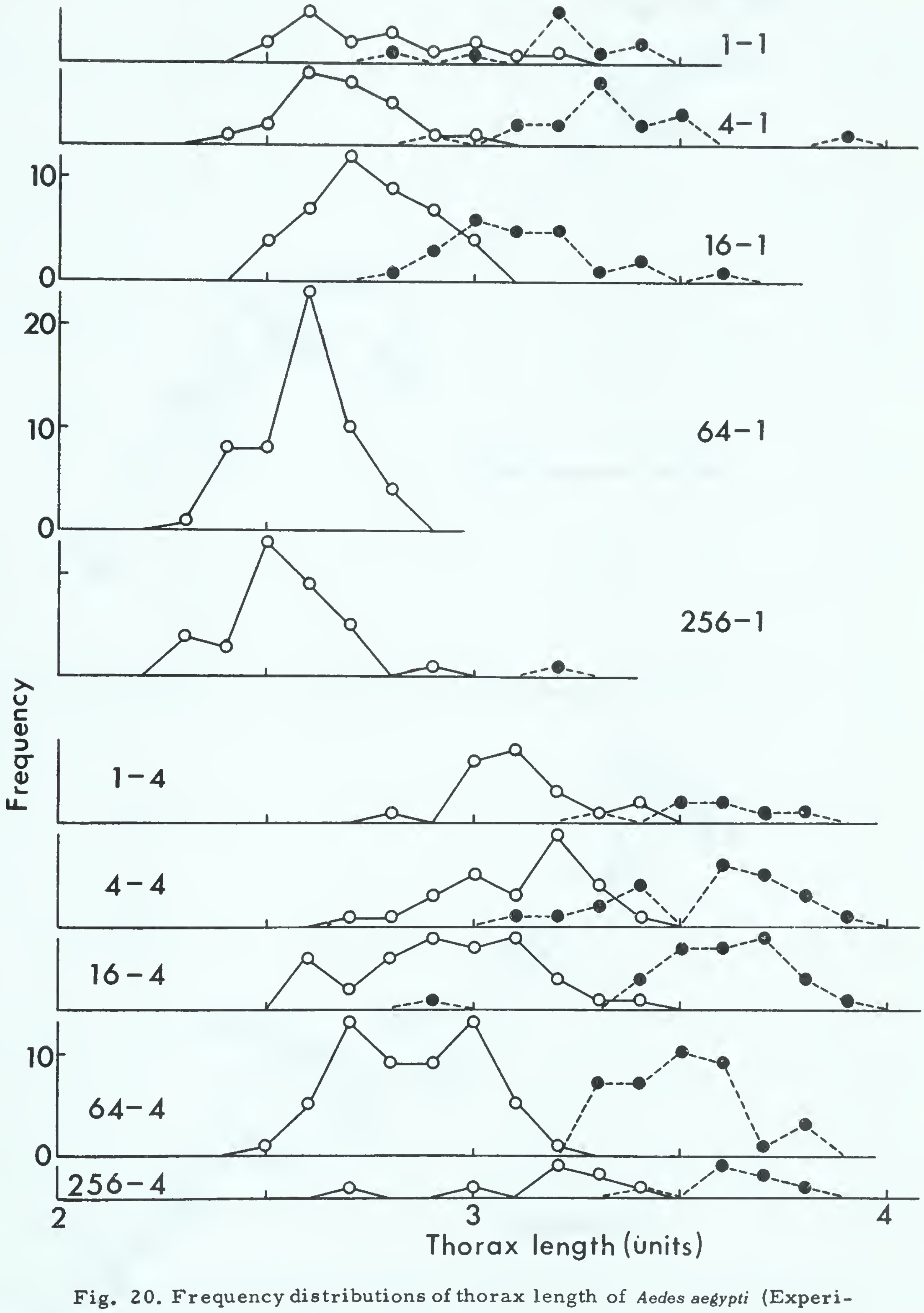
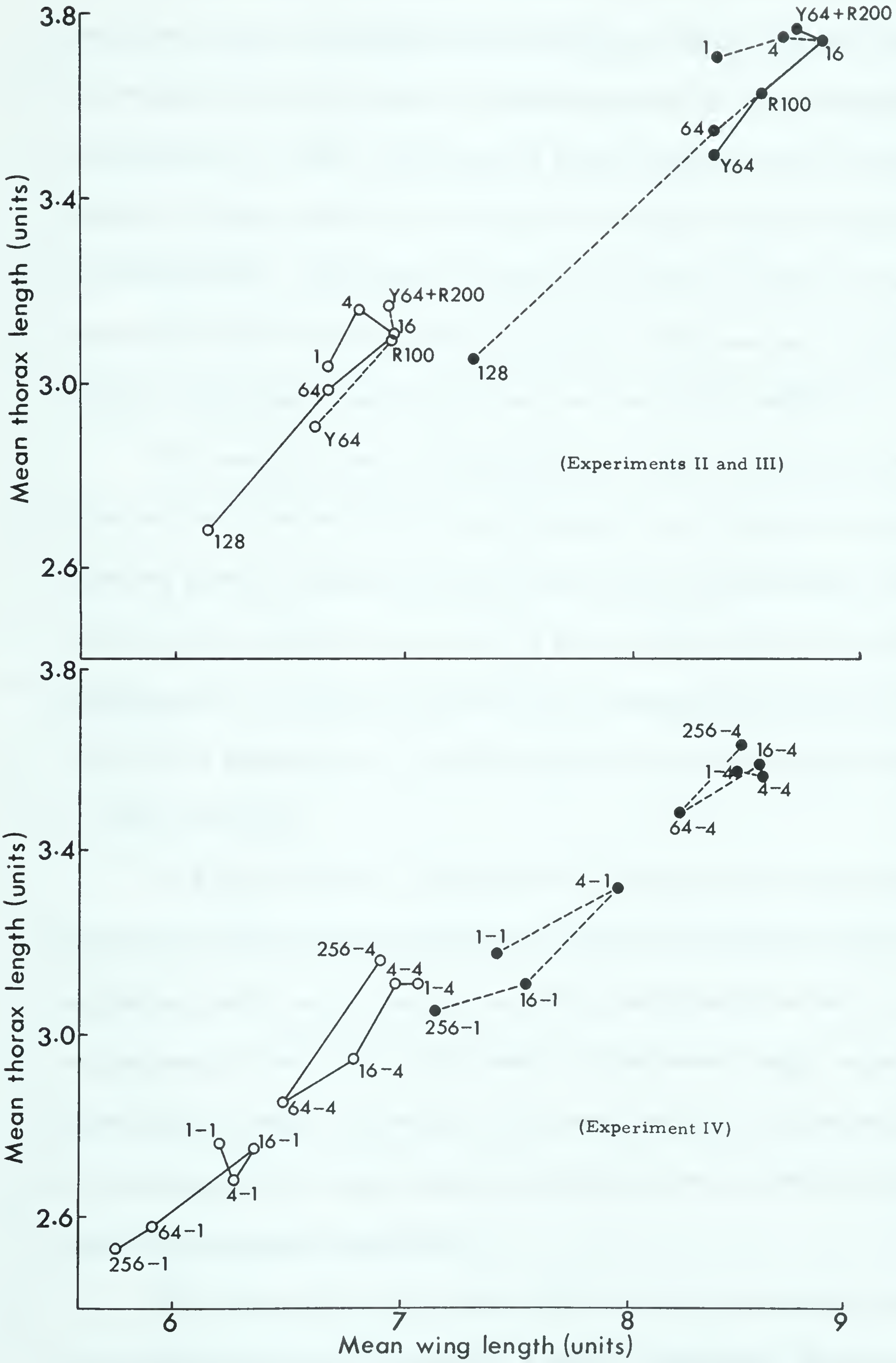


Fig. 20. Frequency distributions of thorax length of *Aedes aegypti* (Experiment IV). 16-4, for example, indicates that the larval density is 16 and the amount of yeast is 4 units per larva. ○ : males; ● : females.



Figs. 21 & 22. The relation between mean thorax length and mean wing length of *Aedes aegypti*. Figure shown indicates larval density in Experiment II, and Y, R, and accompanied figure indicate yeast, rabbit pellets, and their amount in units. In Experiment IV, 16-4 for example, indicates larval density - yeast units. O : males; ● : females.

increasing density. However, in densities lower than 16, decreased wing length and rather unchanged thorax length are shown, that is, the points for density levels of 1 and 4 are situated above the line through the points for density 16 to 128. The larvae in lower density receive relatively large amounts of food, because the amount of food per cup was kept constant in this experiment. Therefore, it may be said that at these low density levels the adults resulting from favorable conditions have relatively shorter wing length than those from less favorable conditions.

The same is seen in Experiment III, where different diets were given to the larvae with the same density of 16. The point for the adults from the culture containing yeast 64 units plus rabbit pellets 200 units, which is more suitable than yeast 64 plus rabbit pellets 100 used in Experiment II, is situated above the line through the points for density 16 to 128 in Experiment II, and the point for the less suitable diet, yeast 64, below the line.

In Experiment IV, the situation becomes more complicated, because the experiment consisted two series of constant amount of food per larva, and it is not easy to say which combination of larval density and amount of food is more favorable for the larval stage, especially at lower density levels. However, it is seen that the relative wing length to thorax length for larger amounts of food or lower density of larvae tends to be smaller than others.

The adults from the culture with density 1 and yeast 4 per cup are not considered to have a relatively smaller wing length than other densities, unlike Experiment II. This is perhaps because of the fact that the

food of yeast 4 in Experiment IV is apparently less favorable than that of yeast 64 plus rabbit pellets 100. The adults with relatively small wing length from low larval density seems to appear only when the amount of food is large.

4. CONCLUSION

The results obtained are summarized in Table 8. The density in this table is used in a relative sense to food quantity. Actual density differs according to the amount of food.

High larval density apparently has detrimental effects on the mosquito. Interesting is the relation between very low and low densities. The characteristics seem to indicate that the adults from very low larval density have a slightly reduced flight ability in comparison with those from less low density, as far as judged from the relative wing length. However, repeated experiments are desired, as the number of mosquitoes used in lower densities was not very large.

5. CONSIDERATIONS ON THE MANNER IN WHICH LARVAL DENSITY PRODUCES ITS EFFECTS

From the preceding sections, the effect of larval density is apparent, but its process was not particularly investigated. Since no effects of metabolic wastes of larvae have been demonstrated (Bar-Zeev, 1957; Shannon and Putnam, 1934), high larval density seems to influence the mosquitoes through the stimulation of increased mutual contacts.

Shannon and Putnam (1934) stated "DeBuck, Schoute, and

Table 8. Summary of the effects of larval density in *Aedes aegypti*.

Density	Larval mortality	Larval period	Variation in larval period	Sex ratio	Wing length	Thorax length	Relative wing length to thorax
Very low	Low	Short	Small	Normal	Large	Large	Small
Low	Very low	Very short	Small	Normal	Very large	Large	Large
High	High	Long	Large	♂>♀	Small	Small	Large

Swellengrebel (1932) claim ... that when they (anopheline larvae) live in overcrowded conditions food may remain undigested in the alimentary tract from 12 to 24 hours ... Improper nourishment due to massing habits of the larvae (of Aedes aegypti) may account for this (phenomena at high larval density) ...". However, the situation seems to be more complex than Shannon and Putnam (1934) thought, and neuro-physiological processes may be involved.

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